POSTNATAL DEVELOPMENT OF PORCINE SKELETAL MUSCLE

A.S. DAVIES

B.V.Sc. (Queensland)

A Thesis presented for the Degree

of Doctor of Philosophy in the

University of Edinburgh

1973

ABSTRACT OF THESIS

Name of Candidate A.S. Davies

Address Department of Anatomy, Royal (Dick) School of Veterinary Studies, Edinburgh EH9 1QH

Degree Ph.D. *Date* 20th February 1973.

Title of Thesis Postnatal Development of Porcine Skeletal Muscle

The manner in which skeletal muscle of the pig adapts to meet the changing mechanical and metabolic demands of an increase in body weight during postnatal growth was studied using two different methods.

(1) Histochemical profiles of individual muscle fibres were established using the myosin ATPase, succinate dehydrogenase (SDHase) and glycogen phosphorylase (GPase) reactions. Samples of longissimus and diaphragm muscles from a series of 18 Large White pigs between birth and 60 kg liveweight, and from a series of 16 Large White pigs of mean liveweight of 93 kg, were used.

In both muscles, the number of fibres low in myosin ATPase activity increases with growth, and these fibres are grouped into bundles. In m. longissimus, the estimates of the total fibre population and the number of myosin ATPase low bundles in a complete transverse section of the muscle remain constant, while the mean number of myosin ATPase low fibres per bundle increases from one at birth to 3·2 at 93 kg liveweight. Whereas the complete transverse sectional area of the muscle increases in proportion to the 2/3 power of the body weight, the area occupied by myosin ATPase low fibres increases in direct proportion to the body weight. This observation suggests the mechanism by which larger animals are supported without a relative increase in their muscle mass. Some histochemical evidence was obtained that this is achieved by a transformation of the physiological properties of certain fibres.

The diaphragm of smaller pigs contains a greater proportion of a myosin ATPase high, SDHase high and GPase low fibre type than that of more mature pigs. After initial neonatal differentiation, the muscles studied do not change their proportion of SDHase high fibres during growth.

In both longissimus and diaphragm, the mean transverse sectional area of myosin ATPase high fibres is greatest when the SDHase activity is low. Also, the mean transverse sectional area of SDHase high fibres is greatest when the myosin ATPase activity is low, but this difference is significant only for the diaphragm.

(2) Eighteen female pigs of both the Pietrain and Large White breeds, from birth to 72 kg liveweight, were dissected, and the major tissues were weighed. The growth of fat, muscle and bone, relative to carcass growth, were compared for both breeds. Fat is the fastest developing tissue; fat and muscle grow at a rate higher, and bone at a rate lower, than overall carcass growth. The muscle:bone ratio increases during the growth interval studied.

Growth changes in the distribution of muscle and bone were studied by weighing these tissues after separation into 93 muscle units and 10 bone units. The pattern of growth of both these tissues can be described as an increasing craniocaudal gradient axially and an increasing distoproximal gradient in the limbs. It is proposed that these gradients are an adaption to enhance the animal's propulsive force. The cardiac muscle:skeletal muscle ratio decreases with growth.

Of the/

Use other side if necessary.

Of the major tissues, only the growth of bone is significantly different between the two breeds; this tissue develops faster relative to carcass growth in the Large White. When the breeds are compared at the same body weight, the weight of muscle is greater in the Pietrain over the entire growth range studied. Muscle:bone ratios, compared at the same values of total muscle plus bone, are higher for the Pietrain. There are no significant differences between the muscle:bone ratios of the Large Whites used in the present study and the Large Whites dissected by McMeekan over 30 years ago.

A comparison of the difference in tissue distribution between the two breeds shows that growth gradients for muscle are accentuated in the Pietrain. Although the weight of muscle in all regions is higher in the Pietrain at birth, only the weights of abdominal and femoral muscles and m. longissimus are significantly higher in the Pietrain when these weights are compared in pigs of 60 kg body weight. No difference in bone distribution is apparent between the breeds. Consequently, although the ratio of brachial muscle weight to humerus weight is higher for the Pietrain, in pigs of 60 kg body weight the difference between the breeds is very much greater for the ratio of femoral muscle weight to femur weight. At 60 kg body weight, the heart of the Large White is heavier; the cardiac muscle:skeletal muscle ratio of the Large White is higher over the entire growth range studied.

Since the proportions and distribution of the skeletal muscle, cardiac muscle and bone of the Pietrain are an exaggeration of the changes observed in the Large White during growth, it is concluded that the Pietrain is more mature at the same body weight than the Large White, and that a genetic control of muscle distribution is possible.

A study of samples of m. longissimus, removed from the muscle without restraint to contraction, suggests that the greater development of this muscle in the Pietrain is due to relative hypertrophy of a similar number of component fibres. No difference between the breeds is observed in the proportion of fibre types, as determined by the myosin ATPase reaction.

Natura enim simplex est

et rerum causis superfluis

non luxuriat

Isaac Newton, 1686

CONTENTS

ACKNOWLEDGEMENTS

The opportunity for this study arose through the encouragement of Professor A.R. Muir, the facilities offered by the Department of Anatomy, Royal (Dick) School of Veterinary Studies, and a post-graduate scholarship awarded by the Meat and Livestock Commission.

Thanks are due to Dr. W.C. Smith of the University of Newcastle-upon-Tyne, and to Meat and Livestock Commission staff for the supply of material. The carcass dissections were assisted by second year students, Messrs. P.S. Cottrell, J.J.G. Phaff, N.J. Senior and R.H. Thomas, who were supported by grants from the Meat and Livestock Commission and the Birrell Gray Bequest Fund. The Department's technical staff, headed by Mr. E. Roberts, co-operated in many ways, in particular with the collection of animals, the construction of a back-projection apparatus and the preparation of large numbers of photographic plates. Thanks are also due to Mrs. M. MacIvor and her library staff for cheerfully seeking references from unusual sources. The skill of Miss C.F. Aitken as a typist is evident throughout this thesis. Much friendly advice and encouragement was given by Mr. H.M. Gunn and Miss A.F. MacNaughtan.

I am, however, particularly indebted to Professor A.R. Muir for his great interest and understanding, both academic and personal, and to my wife Jenny for her willingness to accompany me through unfamiliar territory on what has been a long and devious, but always interesting, road.

SUMMARY

The manner in which skeletal muscle of the pig adapts to meet the changing mechanical and metabolic demands of an increase in body weight during postnatal growth was studied using two different methods.

(1) Histochemical profiles of individual muscle fibres were established using the myosin ATPase, succinate dehydrogenase (SDHase) and glycogen phosphorylase (GPase) reactions. Samples of longissimus and diaphragm muscles from a series of 18 Large White pigs between birth and 60 kg liveweight, and from a series of 16 Large White pigs of mean liveweight of 93 kg, were used.

In both muscles, the number of fibres low in myosin ATPase activity increases with growth, and these fibres are grouped into bundles. In m. longissimus, the estimates of the total fibre population and the number of myosin ATPase low bundles in a complete transverse section of the muscle remain constant, while the mean number of myosin ATPase low fibres per bundle increases from one at birth to 3·2 at 93 kg liveweight. Whereas the complete transverse sectional area of the muscle increases in proportion to the 2/3 power of the body weight, the area occupied by myosin ATPase low fibres increases in direct proportion to the body weight. This observation suggests the mechanism by which larger animals are supported without a relative increase in their muscle mass. Some histochemical evidence was obtained that this is achieved by a transformation of the physiological properties of certain fibres.

The diaphragm of smaller pigs contains a greater proportion of a myosin ATPase high, SDHase high and GPase low fibre type than that of more mature pigs.

After initial neonatal differentiation, the muscles studied do not change their proportion of SDHase high fibres during growth.

In both longissimus and diaphragm, the mean transverse sectional area of myosin ATPase high fibres is greatest when the SDHase activity is low. Also, the mean transverse sectional area of SDHase high fibres is greatest when the myosin ATPase activity is low, but this difference is significant only for the diaphragm.

(2) Eighteen female pigs of both the Pietrain and Large White breeds, from birth to 72 kg liveweight, were dissected, and the major tissues were weighed. The growth of fat, muscle and bone, relative to carcass growth, were compared for both breeds. Fat is the fastest developing tissue; fat and muscle grow at a rate higher, and bone at a rate lower, than overall carcass growth. The muscle:bone ratio increases during the growth interval studied.

Growth changes in the distribution of muscle and bone were studied by weighing these tissues after separation into 93 muscle units and 10 bone units. The pattern of growth of both these tissues can be described as an increasing craniocaudal gradient axially and an increasing distoproximal gradient in the limbs. It is proposed that these gradients are an adaption to enhance the animal's propulsive force. The cardiac muscle:skeletal muscle ratio decreases with growth.

Of the major tissues, only the growth of bone is significantly different between the two breeds; this tissue develops faster relative to carcass growth in the Large White. When the breeds are compared at the same body weight, the weight of muscle is greater in the Pietrain over the entire growth range studied. Muscle:bone ratios, compared at the same values of total muscle plus bone, are higher for the Pietrain. There are no significant

differences between the muscle:bone ratios of the Large Whites used in the present study and the Large Whites dissected by McMeekan over 30 years ago.

A comparison of the difference in tissue distribution between the two breeds shows that growth gradients for muscle are accentuated in the Pietrain. Although the weight of muscle in all regions is higher in the Pietrain at birth, only the weights of abdominal and femoral muscles and m. longissimus are significantly higher in the Pietrain when these weights are compared in pigs of 60 kg body weight. No difference in bone distribution is apparent between the breeds. Consequently, although the ratio of brachial muscle weight to humerus weight is higher for the Pietrain, in pigs of 60 kg body weight the difference between the breeds is very much greater for the ratio of femoral muscle weight to femur weight. At 60 kg body weight, the heart of the Large White is heavier; the cardiac muscle:skeletal muscle ratio of the Large White is higher over the entire growth range studied.

Since the proportions and distribution of the skeletal muscle, cardiac muscle and bone of the Pietrain are an exaggeration of the changes observed in the Large White during growth, it is concluded that the Pietrain is more mature at the same body weight than the Large White, and that a genetic control of muscle distribution is possible.

A study of samples of m. longissimus, removed from the muscle without restraint to contraction, suggests that the greater development of this muscle in the Pietrain is due to relative hypertrophy of a similar number of component fibres. No difference between the breeds is observed in the proportion of fibre types, as determined by the myosin ATPase reaction.

1.0 GENERAL INTRODUCTION

The carnivore owes its existence to the evolution in animals of a contractile apparatus based on an ability to develop a mechanical force between protein filaments. A large proportion of the world's animal population demands this machine as a food. It provides more protein for human consumption than the nutritional secretions of animals (Autret, 1970), and is nutritionally of higher quality than plant tissues. This fortuitous link between muscle as a nutritious food source for man, and muscle as a biological machine for the conversion of chemical into mechanical energy, was the subject of a recent symposium (Briskey, Cassens & Marsh, 1970). However, the following examples illustrate how this double role has been overlooked in the study of the development of muscle as a food.

The Cambridge School describe differential growth of muscle throughout the body as a "pattern of early onset of high growth intensity near the extremities passing with an increasing growth rate backwards and upwards to the lumbar region" (Pálsson, 1955). Fowler (1968) and Fowler & Livingstone (1972) divide the body of the pig into 'functional units' which conform to this growth pattern. These workers do not, however, provide a functional explanation of why, for instance, the relative growth of the hindlimb is higher than that of the forelimb, why the proximal regions of the limbs have a higher relative growth than the extremities, or why m. longissimus should have the highest, and the head the lowest relative growth of the 'functional units' studied. Butterfield (1964b) criticises this concept of growth gradients, but does not explain the differences between his findings for cattle and those of the Cambridge School for pigs and sheep.

The functional significance of histochemical reactions used in the study of muscle has also been largely ignored. Observations on the histochemical fibre types in muscles of a variety of mammalian species have resulted in systems of classification using colour descriptions (Ogata, 1958), Arabic (Dubowitz & Pearse, 1960b) and Roman (Engel, 1962) numerals, letters of the Modern European (Stein & Padykula, 1962) and Greek (Guth, Samaha & Albers, 1970) alphabets, and combinations of these (Brooke & Kaiser, 1970; Ashmore & Doerr, 1971; James, 1972). Such confusing and artificial classifications are unnecessary if fibres can be characterised by their mechanical and metabolic properties. Evidence will be provided later that individual fibres can be classified according to their intrinsic speed of contraction, and their capacity for aerobic and anaerobic metabolism, by establishing profiles with selected histochemical reactions. If histochemical methods can in this way provide sufficient information about the function of individual muscles, they can establish a functional basis for the differences in growth rate of muscles during postnatal development. This aspect of histochemistry as a tool for meat research was not envisaged in a review of the subject by Cassens & Cooper (1971). Experiments examining the effect of environment and breed on fibre types, suggested by these workers to be relevant to the processing of meat, will be better planned once the functional significance of the histochemical properties of muscle have been adequately investigated.

Dubowitz (1970) reviews the histochemical studies that have been made on developing muscle. Although presented as part of a symposium on muscle as a food (Briskey, Cassens & Marsh, 1970), most of the observations he reports can be ascribed to the effect of the environmental change from a maternal dependent to an independent existence. In the context of meat production, the relevance

of this change is slight compared with that of subsequent development in which an animal's genetic potential and influences on muscle such as body size, nutrition and sex hormones interact. The use of methods capable of comparing the metabolism, mechanical properties and size of muscle fibres during postnatal growth, when man can control the environment, should elucidate the manner in which breed and environment influence muscle development.

This thesis combines observations on two aspects of the postnatal development of porcine muscle.

(1) Changes in the histochemical properties of two muscles, longissimus and diaphragm, of the Large White pig are studied from birth to commercial slaughter weight at 93 kg liveweight. A hypothesis that the mechanical and metabolic properties of muscle fibres adapt to the changing requirements of an increasing body size is examined. The relationship between the histochemical properties and the transverse sectional area (TSA) of muscle fibres is also studied.

(2) The growth of individual muscles of two breeds of pigs, Large White and Pietrain, is studied from birth to 72 kg liveweight by a total carcass dissection method. The following hypotheses are examined:

(a) The effect of increasing body size on the distribution of muscle in the carcass is satisfactorily described as a growth gradient.

(b) Man can influence the distribution of muscle throughout the carcass by genetic means.

(c) The genetic change so produced is expressed phenotypically by changes in the mean fibre transverse sectional area (TSA), the

total fibre population, and the histochemical properties of muscles, as demonstrated in a particular muscle, m. longissimus.

The two aspects of the work are presented as separate reports. Methods by which they could be combined experimentally to provide a functional explanation of the manner in which body size and breed affect muscle distribution are suggested in a General Discussion. A comparative study between species of body size dependent histochemical properties of the diaphragm, published in collaboration with H.M. Gunn, is included as Appendix 3. This was the subject of a paper presented at the July 1971 meeting of the Anatomical Society of Great Britain and Ireland in Edinburgh (Davies & Gunn, 1971). The results of Part 1 of this thesis were presented at the April 1972 meeting of this Society in Oxford (Davies, 1972a), and have been accepted for publication by the Journal of Anatomy (Davies, 1972b).

2.0 Part 1: POSTNATAL CHANGES IN HISTOCHEMICAL FIBRE TYPES

2.1 INTRODUCTION

When the innervation of fast- and slow-twitch crural muscles of the cat (Buller, Eccles & Eccles, 1960a), guinea-pig (Robbins, Karpati & Engel, 1969) and rat (Close, 1969) is exchanged, the ratio of the intrinsic speeds of contraction of these muscles is reversed. Contraction speed is directly related to the activity of myosin adenosine triphosphatase (myosin ATPase) in a variety of muscles (Bárány, 1967), and cross-innervation of fast and slow muscles alters the activity of myosin ATPase demonstrated biochemically (Buller, Mommaerts & Seraydarian, 1969, 1971; Samaha, Guth & Albers, 1970; Bárány & Close, 1971). The proportion of fibres shown histochemically to be high in myosin ATPase activity is related to the intrinsic speed of contraction of a muscle (Edgerton & Simpson, 1969), and following cross-innervation there is a change in this histochemical reaction. Thus, when axons normally supplying fast-twitch muscles are made to innervate the slow-twitch soleus muscle of the rabbit (Dubowitz, 1967), guinea-pig (Karpati & Engel, 1967a; Robbins, Karpati & Engel, 1969), cat and rat (Guth, Samaha & Albers, 1970), the proportion of myosin ATPase high fibres in soleus is increased. Similarly, when the nerve to soleus is made to innervate a fast-twitch muscle, there is a decrease in the proportion of myosin ATPase high fibres in the flexor hallucis longus muscle of the cat (Dubowitz, 1967; Guth, Samaha & Albers, 1970) and the flexor digitorum longus muscle of the rabbit (Dubowitz, 1967). The type of innervation therefore has a direct influence on the contractile properties of the muscle fibre. As a corollary, any change in the speed of contraction of a muscle

and the proportion of its fibres with high myosin ATPase activity could be the result of an innervation change.

However, the contractile properties of a muscle can be changed without direct interference with the nerve supply. When the amount of isometric exercise in a muscle is increased by eliminating the effect of synergistic muscles or by impairing the function of the opposite limb, there is a decrease in the speed of contraction (Vrbová, 1963; Lesch, Parmley, Hamosh, Kaufman & Sonnenblick, 1968; Olson & Swett, 1969; Gutmann, Schiaffino & Hanzlíková, 1971). Gutmann & Hájek (1971) show that the ATPase activity of myosin extracted from the extensor digitorum longus muscle of the rat decreases when the tibialis cranialis muscle has been tenotomised for 7 days. Ten weeks after excision of the synergists of the soleus and plantaris muscles of the rat, fewer myosin ATPase high fibres are found in these muscles (Guth & Yellin, 1971). The manner in which isometric exercise affects the contractile properties of muscle is unknown, but the evidence from cross-innervation studies given above suggests that work load may influence the type of innervation of individual fibres.

The concept that usage of a muscle may influence the nervous system is relevant to the understanding of adaption of an animal to the environment, and in the interpretation of changes in muscle caused by disease and experimental procedures. In particular, it is relevant to the study of growth of an animal in which the postural muscles must adapt themselves to support, by means of a force proportional to the transverse-sectional area of the muscles or the square of the body length, a weight proportional to the cube of the body length. In addition, we must consider the problem of growth in an animal such as the pig, which runs at approximately the same speed from birth to adult, even though its

body length increases approximately five-fold in this time; the intrinsic speed of contraction of its propulsive muscles must consequently decrease as the pig grows (Hill, 1950).

Part 1 of this thesis describes growth changes in the histochemical profiles of fibres of the longissimus and diaphragm muscles, and provides evidence that the mechanical and metabolic properties of muscles adapt to the changes brought about by an increase in body size. These muscles of the domestic pig were chosen because:

(1) The arrangement of fibre types in porcine muscles forms a pattern which is much less random than in other mammals.

(2) Both muscles permit sampling in a specific region of the muscle in each animal.

(3) The proportion of histochemical fibre types does not vary greatly in the regions near to sites of sampling.

(4) The muscles can be sampled from slaughter-house pigs with minimal mutilation of the carcasses.

(5) M. longissimus has a shape and fibre architecture that enables the measurement of the transverse section of the muscle, and an estimation of its total fibre population.

(6) The two muscles differ greatly in their function, patterns of usage, and proportion of histochemical fibre types.

The probable significance of the three histochemical reactions chosen is discussed on pages 39-45.

2.2 MATERIALS AND METHODS

2.2.1 Sources and initial preparation of material

Pigs of the Large White breed were obtained from two different sources.

Series 1: Eighteen female pigs (Table 1) with liveweights ranging from 1·3 to 60 kg (2 - 214 days of age) were obtained from a herd intensively selected for lean meat production for 6 years by the School of Agriculture, University of Newcastle-upon-Tyne. They were chosen to include as nearly as possible three pigs of each of the following liveweights: 2, 4, 8, 16, 32 and 64 kg. Pigs of 32 and 64 kg liveweight were killed near Newcastle. Samples of the left longissimus muscle from the dorsomedial region at the thoracolumbar junction (Figs. 1, 14), and of the diaphragm from the left costal region, were removed, chilled, and brought to Edinburgh with the carcasses. Smaller pigs were brought alive to Edinburgh where they were killed and eviscerated by a simulated abattoir procedure. Samples were removed as before.

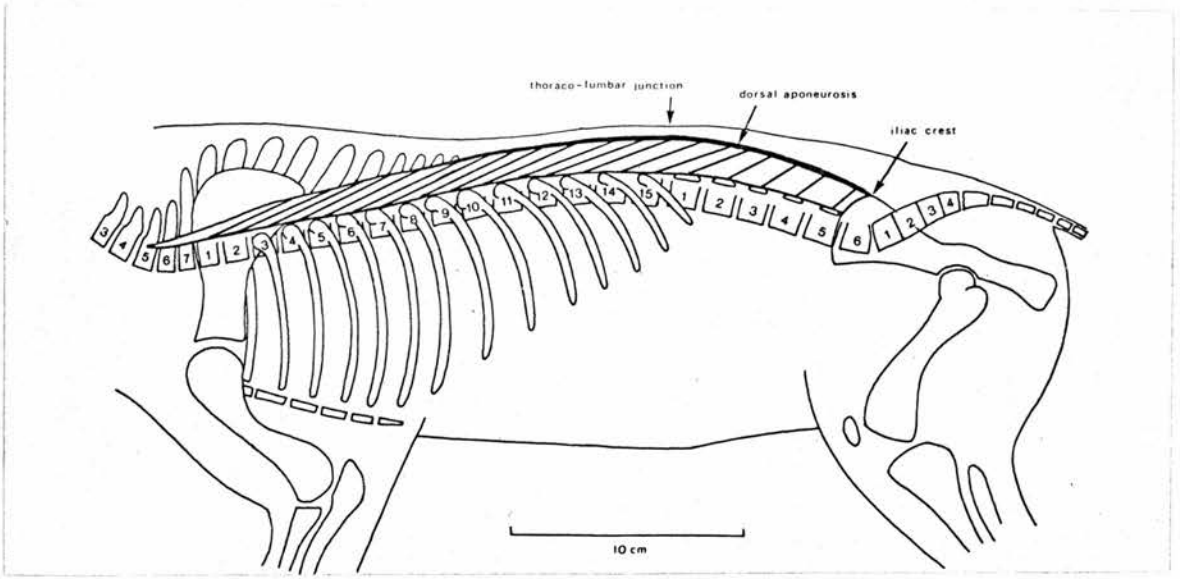


Fig. 1. Diagram of pig, liveweight 6.0 kg, age 21 days, showing the location of m. longissimus and the angles of fibres to the vertebral axis.

Table 1. Measurements on m. longissimus of the pigs of Series 1, and means of measurements on the pigs of Series 2

Pig No.	Live-weight (kg)	Age at slaughter (days)	Weight of m. longiss-imus (g)	TSA of m. longissimus (cm^2)	Depth/width m. longiss-imus	Thoracic and lumbar length (cm)
Series 1						
1	1.27	2	12.6	1.61	0.45	16.0
2	3.69	12	49.5	3.75	0.41	22.6
3	3.72	12	53.3	3.32	0.47	23.7
4	3.98	10	49.4	3.00	0.41	23.9
5	4.16	13	56.8	2.68	0.43	24.1
6	7.33	19	123.0	5.90	0.38	30.5
7	7.69	53	94.8	4.29	0.36	32.9
8	8.11	48	96.2	5.68	0.37	33.6
9	9.46	49	125.0	5.68	0.36	34.8
10	13.0	56	200.0	8.15	0.47	38.8
11	13.5	56	208.0	10.40	0.47	41.6
12	15.0	56	209.0	7.94	0.29	42.1
13	25.0	101	376.0	11.05	0.37	51.3
14	27.8	100	456.0	16.19	0.43	54.1
15	28.9	100	486.0	12.33	0.36	52.6
16	57.4	158	1140.0	35.17	0.60	54.7
17	59.0	214	1091.0	25.74	0.40	54.2
18	59.6	170	990.0	22.09	0.40	62.9
Series 2 (N = 16)						
mean	93.0	183.3	-	33.0	0.70	-
SD	2.8	10.2	-	2.8	0.12	-

Series 2: Ten female and six castrated male pigs with mean liveweight of 93·0 kg (SD = 2·8 kg), carcass weight of 73·6 kg (SD = 3·7 kg), and age of 183 days (SD = 10 days), were sampled at a commercial abattoir at Stirling. These pigs had been reared under test conditions, and came from a variety of herds in which selection for improved carcass conformation was practised. Samples of longissimus and diaphragm were removed from similar regions to Series 1 within 45 minutes of slaughter, chilled, and brought to Edinburgh for processing. All muscle samples were removed pre-rigor and were therefore contracted.

In addition to the above two series of pigs, other pigs of mixed breeding and varying weights were used for studies not directly involved in the measurement of growth changes.

2.2.2. Measurements on m. longissimus

Series 1: The longissimus muscle was dissected from the right side of each pig, cleaned of superficial fat and weighed. An outline of the transverse sectional area (TSA) at the thoracolumbar junction was drawn on paper. This TSA was measured by a paper-weighing method. The distance between the cranial end of the body of the first thoracic vertebra and the caudal end of the body of the sixth lumbar vertebra was measured. The combined length of the thoracic and lumbar vertebrae approximates to, and is proportional to, the length of m. longissimus.

Series 2: The TSA of the muscle was measured after the carcass had been bisected at the thoracolumbar junction. The weight of m. longissimus, and the length of the thoracic and lumbar regions, were not obtained.

2.2.3 Histochemical methods

Blocks of muscle with a TSA of about 1 cm^2 were mounted on a cryostat chuck. A 5 mm thick cork sheet interposed between the chuck and the tissue prevented splitting of the tissue when chuck and tissue were rapidly frozen by plunging into dichlorodifluoromethane (Arcton 12, I.C.I.) cooled to its melting point of -158°C with liquid nitrogen. About ten adjacent serial transverse sections were cut 10 μm thick at -20°C, mounted directly on to coverslips, and allowed to thaw and dry rapidly at room temperature. Histochemical methods were used to demonstrate the activity of three enzymes.

Succinate dehydrogenase (SDHase)

Sections were incubated for 20 min at 37°C in a medium composed of 10 ml of 0·2 M phosphate buffer at pH 7·6, 10 ml of 0·2 M sodium succinate, and 20 ml of nitro blue tetrazolium (1 mg/ml) (Nachlas, Tsou, de Souza, Cheng & Seligman, 1957). Gas bubbles frequently formed between the section and the coverslip; these were often eliminated by drying the section between washing and fixation in 4% formaldehyde.

Glycogen phosphorylase (GPase)

Takeuchi's (1956) modification of the method of Takeuchi & Kuriaki (1955) was used. Sections were incubated for 3 hours at 37°C in a medium consisting of 75 mg glucose-1-phosphoric acid, 15 mg adenosine-5'-monophosphoric acid, 3 mg glycogen, 22·5 ml distilled water, 15 ml of 0·1 M acetate buffer at pH 5·8, one international unit of protamine zinc insulin and 7·5 ml of absolute ethanol. They were subsequently washed, dried, fixed in absolute ethanol, dried, and stained with dilute Lugol's iodine for 3 minutes. Because the colour faded, iodine staining was repeated immediately before subsequent use of the section.

Myosin ATPase

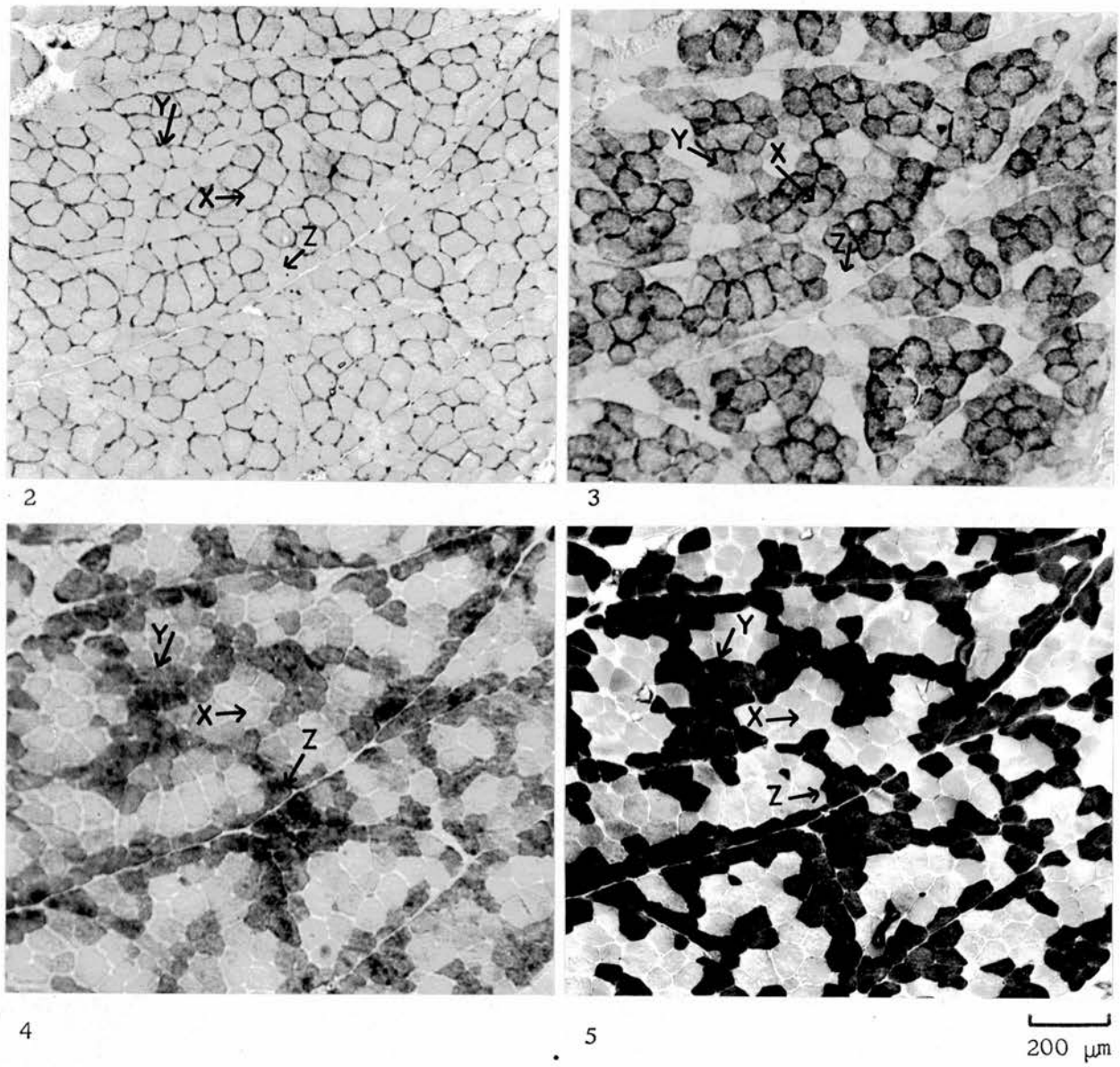
The calcium-cobalt method of Padykula & Herman (1955) was modified to improve the buffering capacity of the medium. Sections were fixed for exactly 2 minutes in cacodylate buffered 4% formaldehyde at pH 7·0. Without fixation, the sections float off the coverslip, and prolonged fixation affects the characteristics of the enzyme (Stein & Padykula, 1962; Guth & Samaha, 1969). Sections were incubated for 20 minutes at 37°C in a freshly made medium consisting of 8 ml of 1·0 M tris-(hydroxymethyl)-aminomethane, 4 ml of 0·18 M calcium chloride, and 60 mg ATP disodium salt made up to 30 ml with distilled water, which was then adjusted to a pH of 9·5 with 0·1 N HCl and made up to a final volume of 40 ml. The final concentration of ATP was therefore 2·4 mM. With two washes in distilled water between treatments, the sections were immersed in 2% cobalt chloride for 3 minutes and developed in dilute ammonium sulphide for 1 minute.

Cell outlines

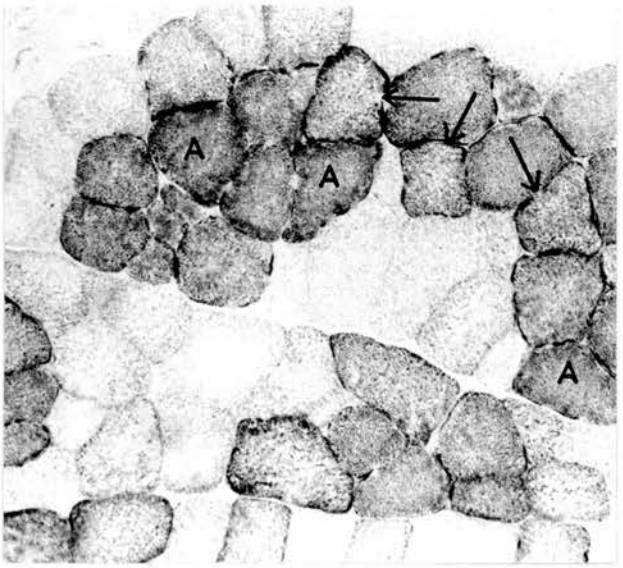
Sections were fixed for 10 minutes in 4% formaldehyde, washed, and stained for 20 minutes in Ehrlich's haematoxylin.

2.2.4 Determination of histochemical profiles of muscle fibres

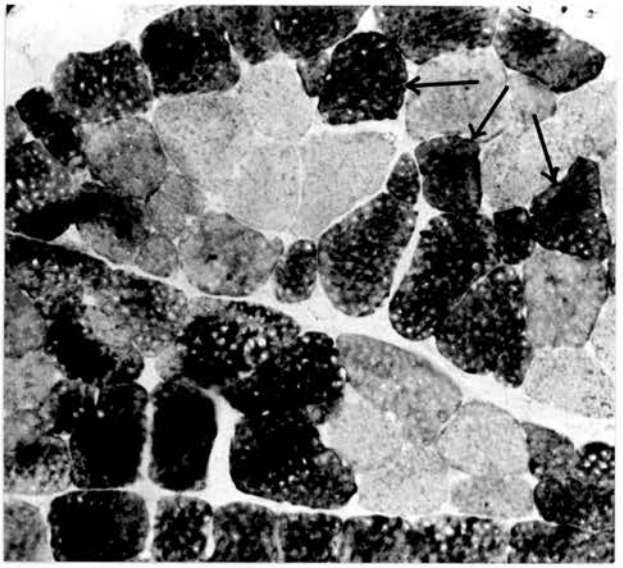
Profiles of about 400 individual fibres in each section were established by first back-projecting a haematoxylin stained section on to a glass screen, enabling a tracing of the fibre outlines to be made on transparent paper. Each serial section was then projected in turn. The histochemical reaction of each fibre was indicated on the tracing; Figs. 2-8 illustrate the type of material used. The numbers of each fibre type so determined were counted, and the proportions were calculated. To estimate the level of enzymes that showed a



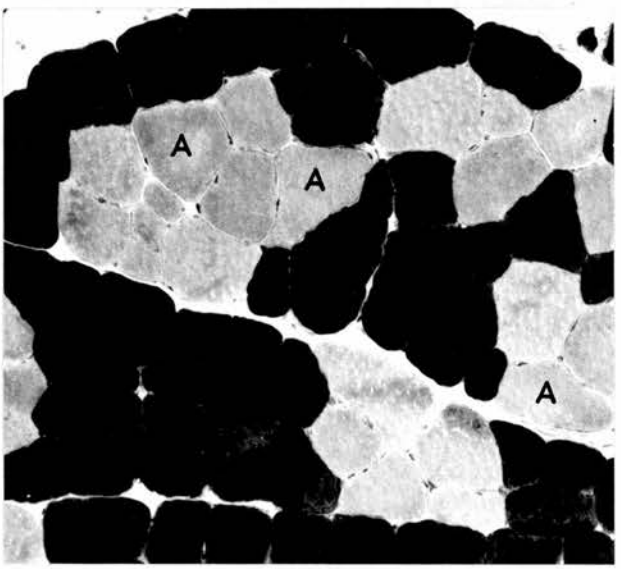
Figs. 2-5. Transverse serial fresh frozen sections of the diaphragm of a Large White pig, liveweight 25 kg, age 101 days, demonstrating fibre outlines with Ehrlich's haematoxylin (Fig. 2), and the activity of SDHase (Fig. 3), GPase (Fig. 4) and myosin ATPase (Fig. 5). X, Y and Z indicate A1,Sh,P1; Ah,Sh,Ph and Ah,S1,Ph fibres respectively (see Table 2).



6



7



8

100 µm

Figs. 6-8. Transverse serial fresh frozen sections of the diaphragm of an adult sow stained for SDHase (Fig. 6), GPase (Fig. 7) and myosin ATPase (Fig. 8). Arrows indicate fibres high in both SDHase and GPase activity. Fibres marked A have low myosin ATPase activity but their SDHase activity is at least as high as that of any myosin ATPase high fibre.

continuous spectrum of activity between fibres, a simple division into 'high' and 'low' was made for each fibre, relative to the overall level of activity of fibres in each section. It was not considered possible to compare one sample with another, because of difficulties of standardisation of the preparation and processing of the material. This is considered to be a source of variation in the quantitative data between samples, and precludes the possibility of a comparison between species based on overall enzyme activity.

2.2.5 Estimation of the mean TSA of muscle fibre types

The areas of paper representing the three main fibre types (myosin ATPase low, SDHase high; myosin ATPase high and SDHase either high or low), prepared as above, were weighed to give a measurement of the proportion of the TSA occupied by each fibre type.

2.2.6 Measurement of the TSA frequency distribution of muscle fibre types

Sections of both longissimus and diaphragm from three pigs of liveweights 4·0, 13 and 98 kg were chosen because they represented characteristic stages in the development of histochemical fibre types. Fibre type profiles, using the myosin ATPase and SDHase reactions only, were made on tracing paper. Individual TSAs of 300 to 400 fibres for each muscle were measured with a compensating planimeter, and frequency polygons for each fibre type were constructed.

2.2.7 Measurements on 'myosin ATPase low bundles' in longissimus

Low power back-projection of sections stained for myosin ATPase enabled a count of the number of myosin ATPase low bundles in an area whose magnification was chosen to include from 30 to 120 bundles. Using the measurement of TSA of the whole muscle, an estimate of the total number of bundles in the TSA was made.

At the same time, the number of myosin ATPase low fibres in each bundle was recorded, and the mean number calculated. In addition, similar measurements were made on several regions of a complete section of longissimus of a 21-day-old, 6·0 kg liveweight, Large White X Landrace female pig, cut transversely to the direction of the muscle fibres at the thoracolumbar junction.

2.2.8 Statistical methods

Differences in the mean values of fibre type proportions between muscles, in estimates of the number of myosin ATPase low bundles in the transverse section of longissimus between groups of pigs, and in the number of myosin ATPase low fibres per bundle between groups of pigs, were tested for significance at the 5% level by Student's t test. The significance of the difference in mean TSA between fibre types was tested on paired data within samples or within pigs at the 5% level by Student's t test. Regression lines, significance of difference between regression coefficients at the 5% level, and analysis of variance were calculated by methods outlined by Diem & Lentner (1970).

2.3 RESULTS

2.3.1 Aspects of the anatomy of longissimus and diaphragm of the pig

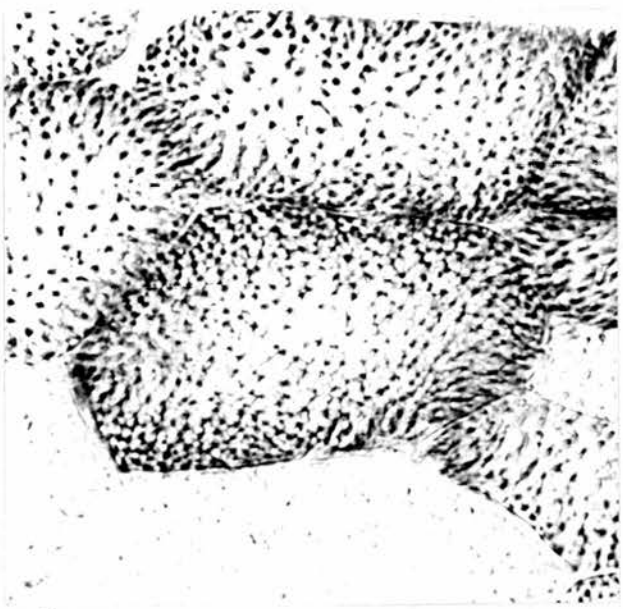
2.3.1.1 Fibre architecture (Fig. 1, page 8)

As in other mammals, fasciculi in the costal diaphragm of the pig run directly to the central tendon from their origins on the ribs and costal cartilages. Fasciculi within m. longissimus thoracis et lumborum of the pig originate on the ribs, transverse processes of the lumbar vertebrae, and intertransverse ligaments. They pass caudally at an angle to the vertebral axis that becomes greater towards the lumbar region (Fig. 1), and laterally at an angle to the sagittal plane of approximately 20^{o} at the thoracolumbar junction, to end on a thick aponeurosis that covers the muscle dorsally before being inserted on to the iliac crest. These large paired muscles, that comprise 4% of the weight of a pig of 60 kg liveweight, are therefore powerful extensors of the thoracic, lumbar and lumbosacral intervertebral joints, and provide a propulsive thrust to the pelvic limbs.

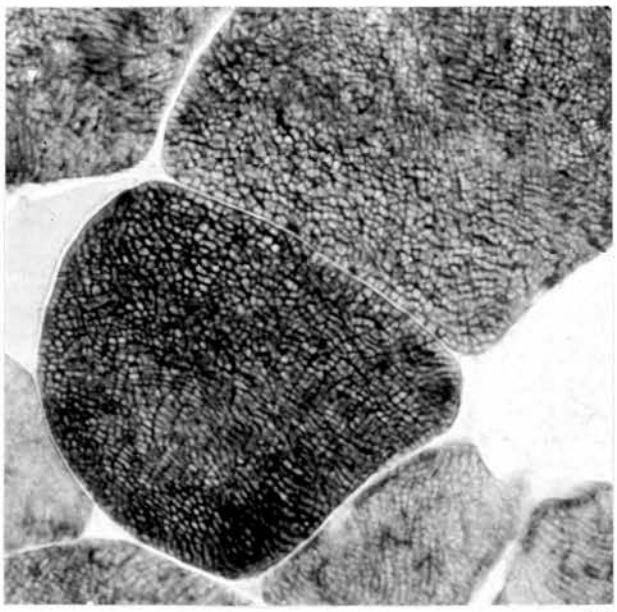
2.3.1.2 Qualitative histochemistry

Succinate dehydrogenase (Figs. 3, 6, 9, 12)

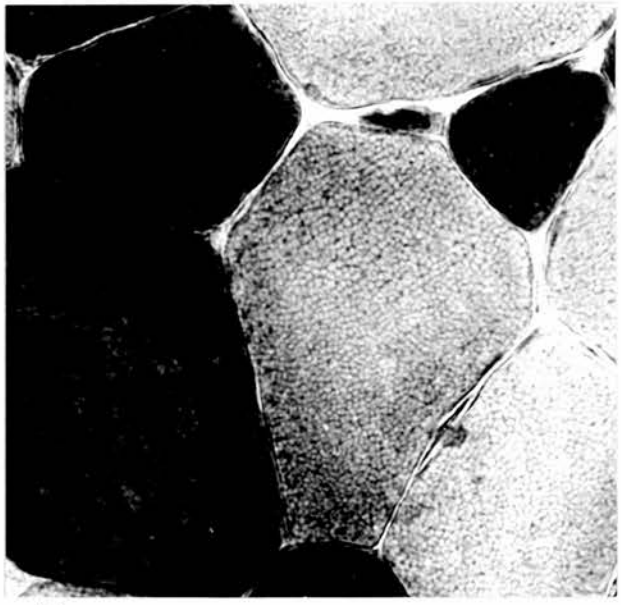
Diformazan deposition occurs as blue dots or irregular areas that appear to form a network around the myofibrils. In fibres with a high level of activity, diformazan deposition is highest in the subsarcolemmal region. It is frequently observed that fibres shown in serial section to be myosin ATPase low have a pattern of intense blue, punctate, clearly defined dots, moderately dense and evenly distributed, whereas the colour of the reaction in ATPase high fibres is purplish, especially in freshly stained sections. This variation in reaction is not sufficiently consistent to use for identifying fibre types, and is not apparent in black and white microphotographs.



9



10



11

50 μm

Fig. 9. Transverse fresh frozen section of m. longissimus of a Large White pig, liveweight 91·2 kg, age 168 days, stained to demonstrate SDHase activity.

Fig. 10. Transverse fresh frozen section of m. pectoralis superficialis of a pig of commercial slaughter weight stained to demonstrate GPase activity.

Fig. 11. Transverse fresh frozen section of the diaphragm of a Large White pig, liveweight 92·5 kg, age 181 days, stained to demonstrate myosin ATPase activity.

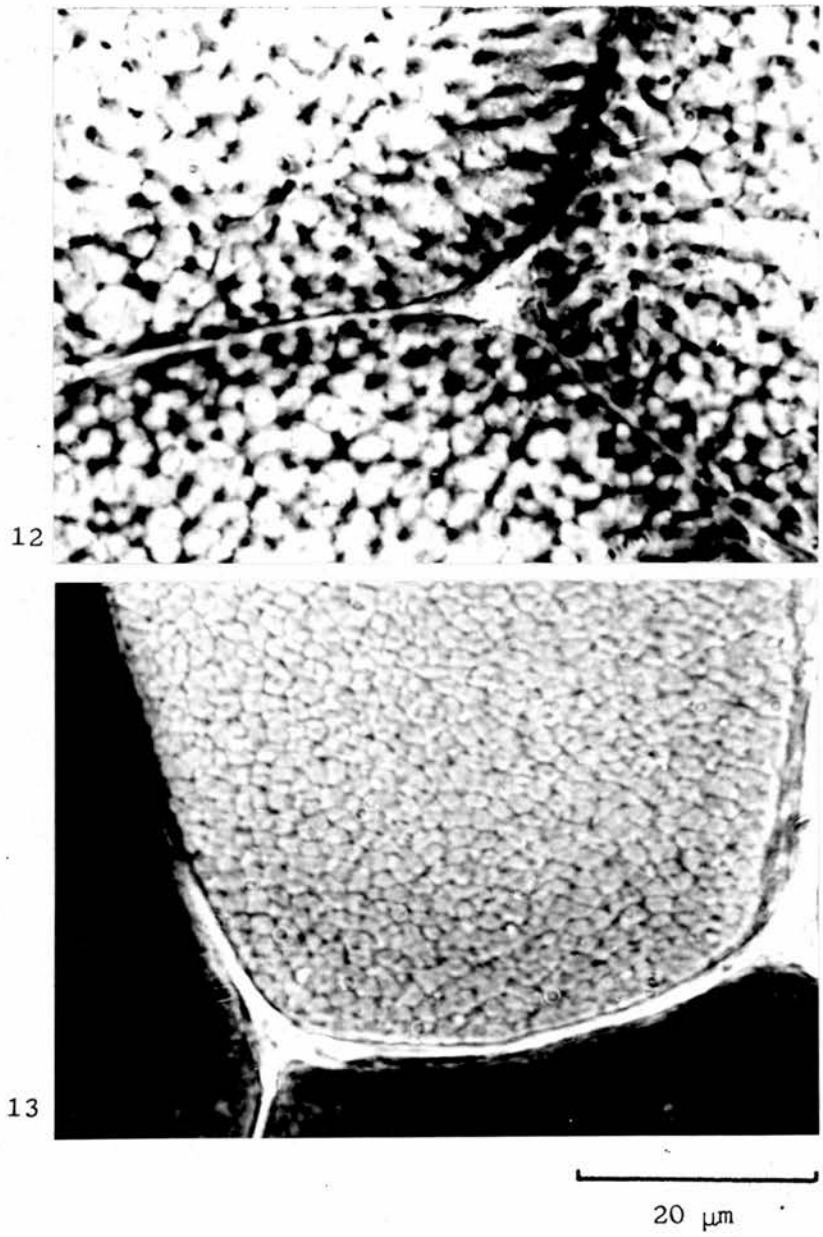


Fig. 12. The section shown in Fig. 9, at a higher magnification.

Fig. 13. The section shown in Fig. 11, at a higher magnification.

Glycogen phosphorylase (Figs. 4, 7, 10)

Fibres vary in reaction from an intense blue network to a paler blue, to a diffuse pink, to fibres coloured only by the iodine.

Myosin ATPase (Figs. 5, 8, 11, 13)

Myosin ATPase high fibres show a dense brown reaction in which a brown network can usually be seen. These are usually distinct from myosin ATPase low fibres in which only the brown network is seen. At certain stages of growth, fibres with an intermediate myosin ATPase reaction are evident (see paragraph 2.3.2.6). Blood vessels are inconsistently stained (Figs. 8, 11). Myosin ATPase low fibres frequently have an activity of SDHase equal to or greater than that of adjacent myosin ATPase high fibres (Figs. 6, 8); the activity of SDHase cannot therefore be graded from the myosin ATPase reaction.

2.3.1.3 Incidence and proportion of histochemical fibre types (Tables 2, 3)

In both longissimus and diaphragm, six types of muscle fibre can be identified by establishing profiles with the three histochemical reactions (Table 2). No fibres low in both myosin ATPase and SDHase activity are observed. The GPase reaction within individual fibres corresponds to the myosin ATPase reaction in all except 10% of fibres in the longissimus and 13% of fibres in the diaphragm of the pigs of Series 2; those which do not correspond are of three types, none of which exceeds 5% of the total fibre population or differs significantly in proportion between the two muscles. In both muscles, regions were frequently seen where the incidence of GPase high fibres was reduced. In one sample of longissimus, these regions were too extensive for the GPase reaction to be used to determine fibre profiles. This sample has been excluded from the data in Table 2.

Table 2. Longissimus and diaphragm; Series 2.
Mean proportions of fibre types based on three histochemical reactions.

Histochemical reaction h = high 1 = low			longissimus 15 pigs; 5763 fibres		diaphragm 16 pigs; 7299 fibres		Significance of Difference
myosin ATPase	SDHase	GPase	mean %	SD	mean %	SD	
A1	Sh	Ph	2·0	3·34	5·0	6·2	N.S.
A1	Sh	P1	15·8	6·0	31·5	6·8	P< ·001
Ah	Sh	Ph	13·9	5·9	41·0	7·4	P< ·001
Ah	Sh	P1	3·0	4·8	4·2	3·0	N.S.
Ah	S1	Ph	60·3	9·0	14·1	7·3	P< ·001
Ah	S1	P1	5·0	7·2	4·2	5·0	N.S.

Table 3. Longissimus and diaphragm; Series 2.
Mean proportion of fibre types based on two histochemical reactions.

Histochemical Reaction h = high 1 = low		longissimus 16 pigs; 6151 fibres		diaphragm 16 pigs; 7299 fibres		Significance of Difference
myosin ATPase	SDHase	mean %	SD	mean %	SD	
A1	Sh	18·0	4·5	36·5	5·9	P< ·001
Ah	Sh	17·3	4·0	45·2	7·4	P< ·001
Ah	S1	64·7	4·8	18·3	7·3	P< ·001

Fibres in which the myosin ATPase and GPase reactions correspond occur as three types; each exceeds 10% of the total fibre population and differs significantly ($P < 0{\cdot}001$) in proportion between the two muscles. When only the myosin ATPase and SDHase reactions are used to determine profiles, the standard deviation of these three fibre types is either reduced or remains constant (Table 3). Because the incorporation of the GPase reaction into a classification of muscle fibres introduces a slight variability that could confuse differences between muscles, many of the subsequent results use classifications under the three main fibre types shown in Table 3.

2.3.1.4 Comparison of the mean TSA of three types of fibres (Table 4)

In both muscles, the coefficient of variance of the TSA is lowest for the ATPase low, SDHase high (Al,Sh) fibre type. The mean TSA of fibres high in myosin ATPase activity is significantly greater ($P < 0{\cdot}001$) when the SDHase activity is low. Fibres high in SDHase activity have a greater TSA when the myosin ATPase activity is low; the difference is significant in the diaphragm ($P < 0{\cdot}02$) but not significant in longissimus.

A comparison of fibre TSA between the two muscles shows that the mean TSA of all fibres is significantly greater ($P < 0{\cdot}01$) in longissimus. Since the mean TSA of Al,Sh and ATPase high, SDHase high (Ah,Sh) fibres is not significantly different between the muscles, this difference must be mainly due to the significantly greater ($P < 0{\cdot}05$) mean TSA of the ATPase high, SDHase low (Ah,Sl) fibres.

Table 4. Longissimus and diaphragm; Series 2.
Mean TSA of all fibres sampled, of myosin ATPase low fibres (Al, Sh), and of fibres myosin ATPase high and either SDHase high (Ah, Sh) or low (Ah, Sl).

Fibre Type	Longissimus			Diaphragm		
	Mean TSA (μm^2)	SD	Coef. of variance	Mean TSA (μm^2)	SD	Coef. of variance
All fibres	5800	1100	19%	4500	1100	24%
Al,Sh	4700	900	19%	4600	900	20%
Ah,Sh	4500	1100	24%	4100	1200	29%
Ah,Sl	6400	1400	22%	5200	1600	31%

2.3.1.5 Organisation of histochemical fibre types (Figs. 3-8, pages 13, 14)

There is a greater degree of organisation of fibre types in porcine muscle than in other species. In the longissimus and diaphragm, one or more bundles of fibres characterised by low activity of myosin ATPase and GPase, but high activity of SDHase (Al,Sh,Pl fibres) are located within each fasciculus. In the diaphragm, these 'myosin ATPase low bundles' contain more fibres than in longissimus. Myosin ATPase low bundles are surrounded by a zone of fibres with high activity of myosin ATPase, GPase and SDHase (Ah,Sh,Ph fibres). Elsewhere, the fasciculus is occupied by fibres high in myosin ATPase and GPase, but low in SDHase activity (Ah,Sl,Ph fibres).

2.3.1.6 Distribution of myosin ATPase low (A1) fibres in m. longissimus (Fig. 14)

The number of A1 bundles per mm^2, and the mean number of A1 fibres per bundle, is greater in the medial and lateral regions of the muscle than in the middle region. There appears to be a direct relation between the density of the bundles and the number of A1 fibres per bundle. Samples for the growth study were taken from the dorsomedial region of the muscle; sampling this region may introduce an error due to the variation in distribution of fibre types across the region. The mean number of A1 fibres per bundle in sections of longissimus of the pigs of Series 2 was therefore tested by an analysis of variance. The variance within pigs, between two regions of each section approximately 1 cm apart, is significantly less ($P < 0{\cdot}05$) than the variance between pigs. Therefore it is unlikely that the method of sampling affects the interpretation of the results.

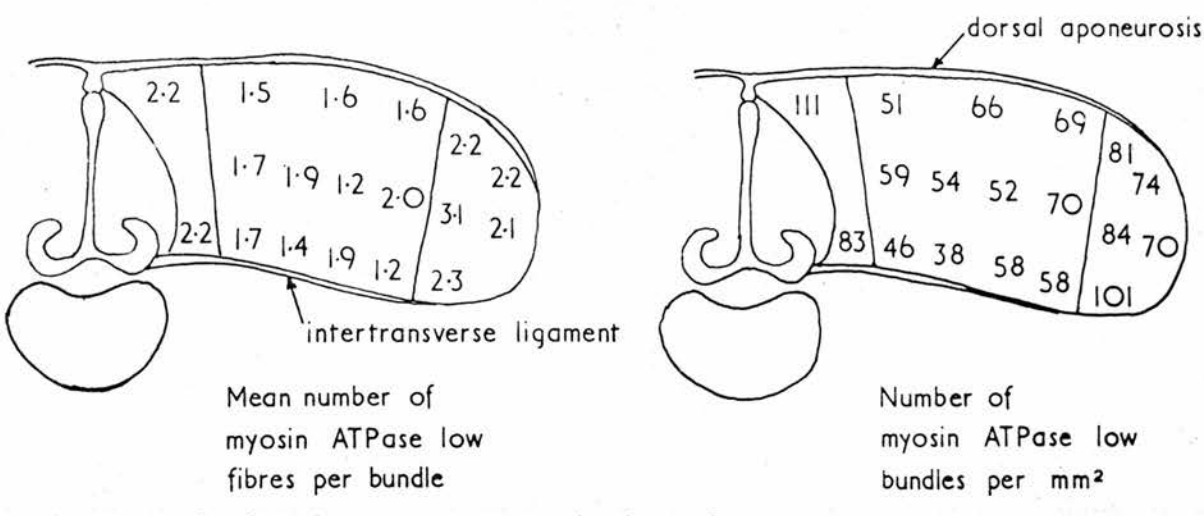


Fig. 14. Variation in the characteristics of myosin ATPase low bundles in a complete transverse section of m. longissimus at the thoracolumbar junction of a pig, liveweight 6·0 kg, age 21 days. The vertical lines separate medial and lateral regions of the muscle containing myosin ATPase low bundles with a mean number of A1 fibres per bundle greater than 2·0, and a density of A1 bundles greater than 70 per mm^2.

2.3.2 Growth changes in longissimus and diaphragm of the Large White pig

2.3.2.1 Relative growth of weight, length and TSA of longissimus (Table 1, Fig. 15)

The measurements of weight of longissimus, TSA of longissimus at the thoracolumbar junction, and the combined lengths of the thoracic and lumbar vertebrae for the pigs in Series 1 are shown in Table 1. These data are plotted as a double logarithmic regression in Fig. 15. The slopes of the two lines suggest that throughout the period of growth studied, the TSA is proportional to the 2/3 power, and the length is proportional to the 1/3 power of the weight of longissimus. The values of the ratio of depth to width of

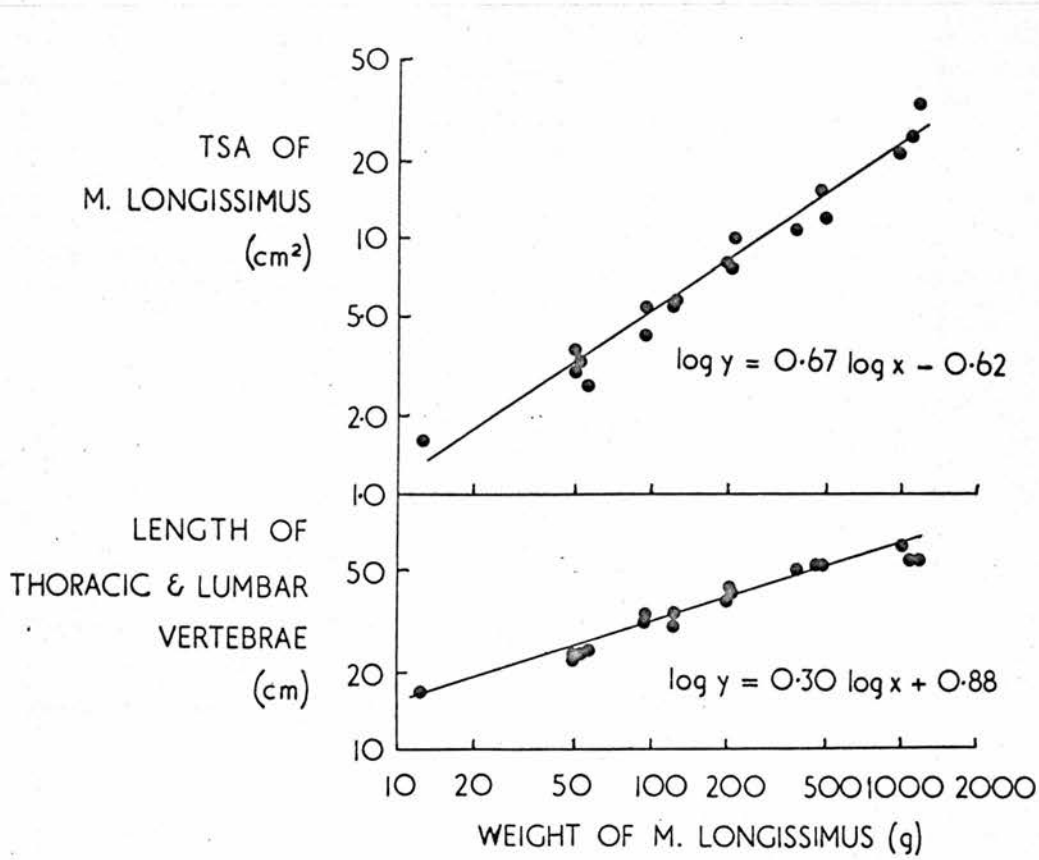


Fig. 15. M. longissimus of 18 Large White pigs (Series 1). Regression of log (TSA at thoracolumbar junction) and log (length of thoracic and lumbar vertebrae) on log (weight of m. longissimus).

the muscle at the thoracolumbar junction do not appear to change with growth in Series 1. The mean ratio is 0·41, with a standard deviation of 0·07. The value of this ratio is significantly higher in Series 2 ($P < 0·001$), possibly because the measurements were made on intact carcasses in Series 2, and on isolated muscles in Series 1.

Since the dimensions of the muscle in Series 1 maintain a constant proportion with one another, and the angles between the fibres in a given region of the muscle and the vertebral axis (as shown in Fig. 1) do not appear to change with growth, it is possible at all stages of growth to relate the mean fibre TSA, as measured on a section cut transversely to the direction of the fibres, to the TSA of the whole muscle cut transversely to the vertebral axis.

2.3.2.2 Growth in mean fibre TSA and total fibre population of m. longissimus (Fig. 16)

The double logarithmic regression of the mean fibre TSA and the TSA of the whole muscle is linear ($r = 0·97$) for all the pigs of both series except one at two days old. Since the regression coefficient of 0·96 (SD = 0·04) is not significantly different from 1, the mean fibre TSA is directly proportional to the TSA of the muscle. The histological appearance of the endomysium and perimysium does not suggest a disproportionate development of tissues other than muscle fibres. After 10 days of age, growth in TSA (and hence weight of the muscle) is therefore accounted for by the growth of a constant population of muscle fibres.

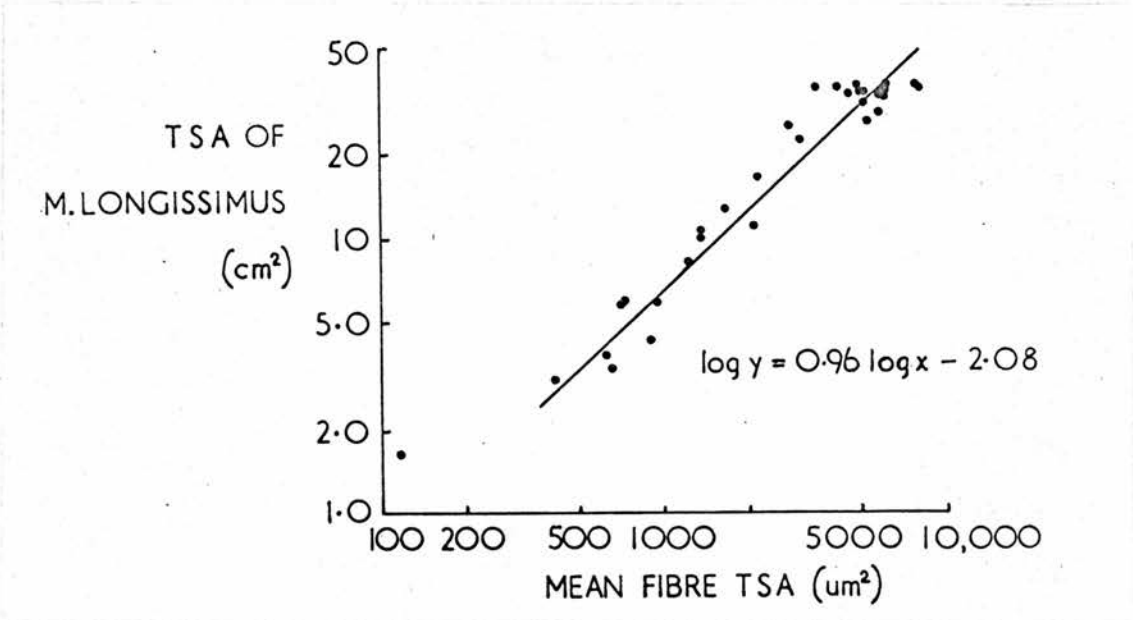


Fig. 16. M. longissimus of 34 Large White pigs (Series 1 and 2). Double logarithmic regression of TSA of the whole muscle on mean fibre TSA at the thoracolumbar junction.

2.3.2.3 Changes in the TSA frequency distribution of three fibre types in longissimus and diaphragm (Fig. 17)

TSA frequency polygons for histochemical fibre types of three pigs are shown for the longissimus and diaphragm in Fig. 17. The frequency intervals for pigs of liveweight 4·0, 13 and 98 kg are 250, 500 and 1000 μm^2 respectively. Since the frequency intervals are represented by the same length of abscissa for each graph, and the frequency is shown as a percentage of fibres sampled, the sum of

the areas under the polygons is the same for each of the six muscles. The area under each polygon indicates the proportion of the muscle occupied by each fibre type. The polygons indicate graphically the differences in the proportion of fibre types discussed in the next paragraph, both between the two muscles and between characteristic growth stages. In addition, they indicate that although there is some relationship between fibre type and TSA, there is considerable overlapping of these properties within individual fibres.

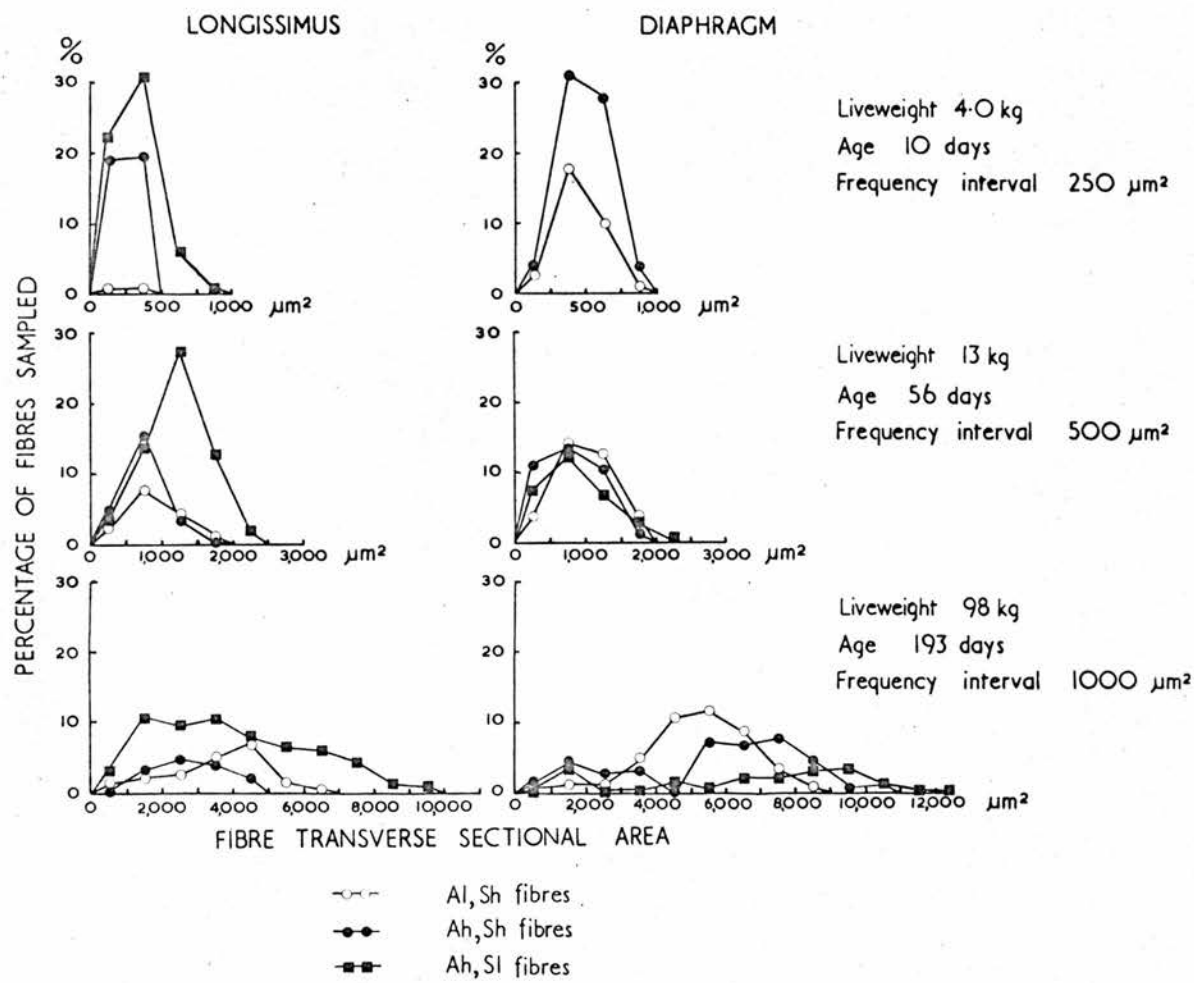


Fig. 17. TSA frequency distribution of three fibre types in longissimus and diaphragm of the Large White pig at three stages of growth. Fibres are classified by the myosin ATPase and SDHase reactions as in Table 3.

2.3.2.4 Changes in the proportion of fibre types in longissimus and diaphragm (Tables 2, 5; Figs. 17-32)

Table 5 shows, for the pigs of Series 1, the proportion of fibres low in activity of each of the enzymes used. The data are derived from a classification of six fibre types for both muscles of each pig, similar to that in Table 2. In both muscles, the proportion of myosin ATPase low fibres increases with increasing body size (Figs. 17-32).

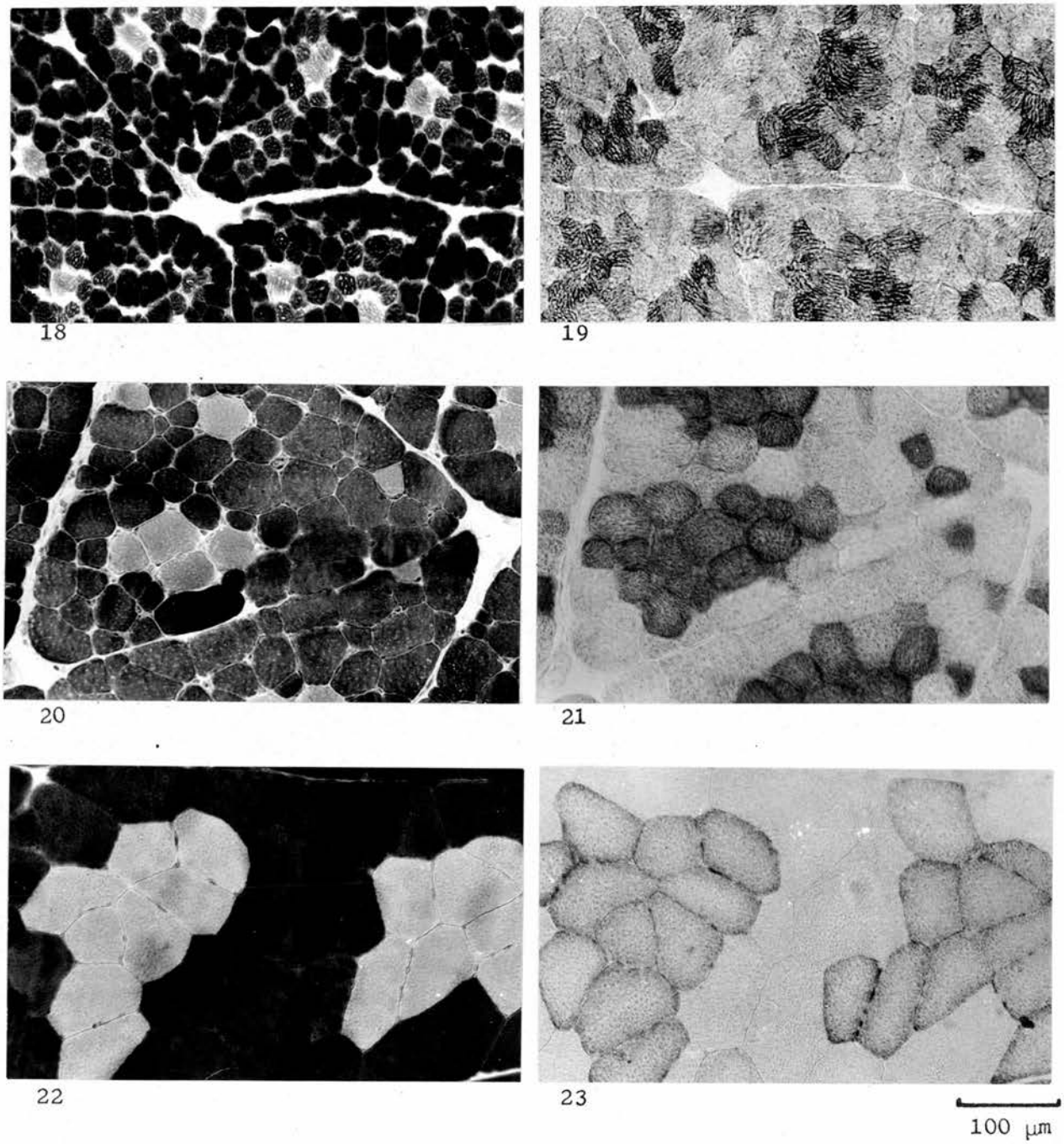
At two days of age, both muscles are composed entirely of SDHase high fibres. Fibres low in the activity of this enzyme are differentiated in both muscles by 12 days after birth. In longissimus, the proportion of SDHase low fibres does not appear to change after this early differentiation process. The diaphragm has a lower proportion of SDHase low fibres in the pigs of Series 2, but since the grading of the reaction between fibres is not as clear-cut in this muscle as in longissimus (Figs. 23, 29), this difference cannot be regarded as significant. It is apparent, however, that in both muscles the proportion of SDHase low fibres does not increase with increasing body size.

At two days, the activity of GPase in all fibres is low. By 10 days of age, the activity of this enzyme has usually developed sufficiently to distinguish fibres with different levels of activity, but before 13 days of age, iodine stains the GPase high fibres pink or purple rather than the blue colour characteristic of the GPase high fibres of more mature pigs. In the diaphragm of the smaller pigs of Series 1, a fibre type in which the GPase and myosin ATPase reactions do not correspond occurs in significant proportions. This is the Ah,Sh,Pl fibre type that averages 7·4% of the total fibre population in 10 pigs from 10 to 56 days of age. This fibre is responsible for the higher

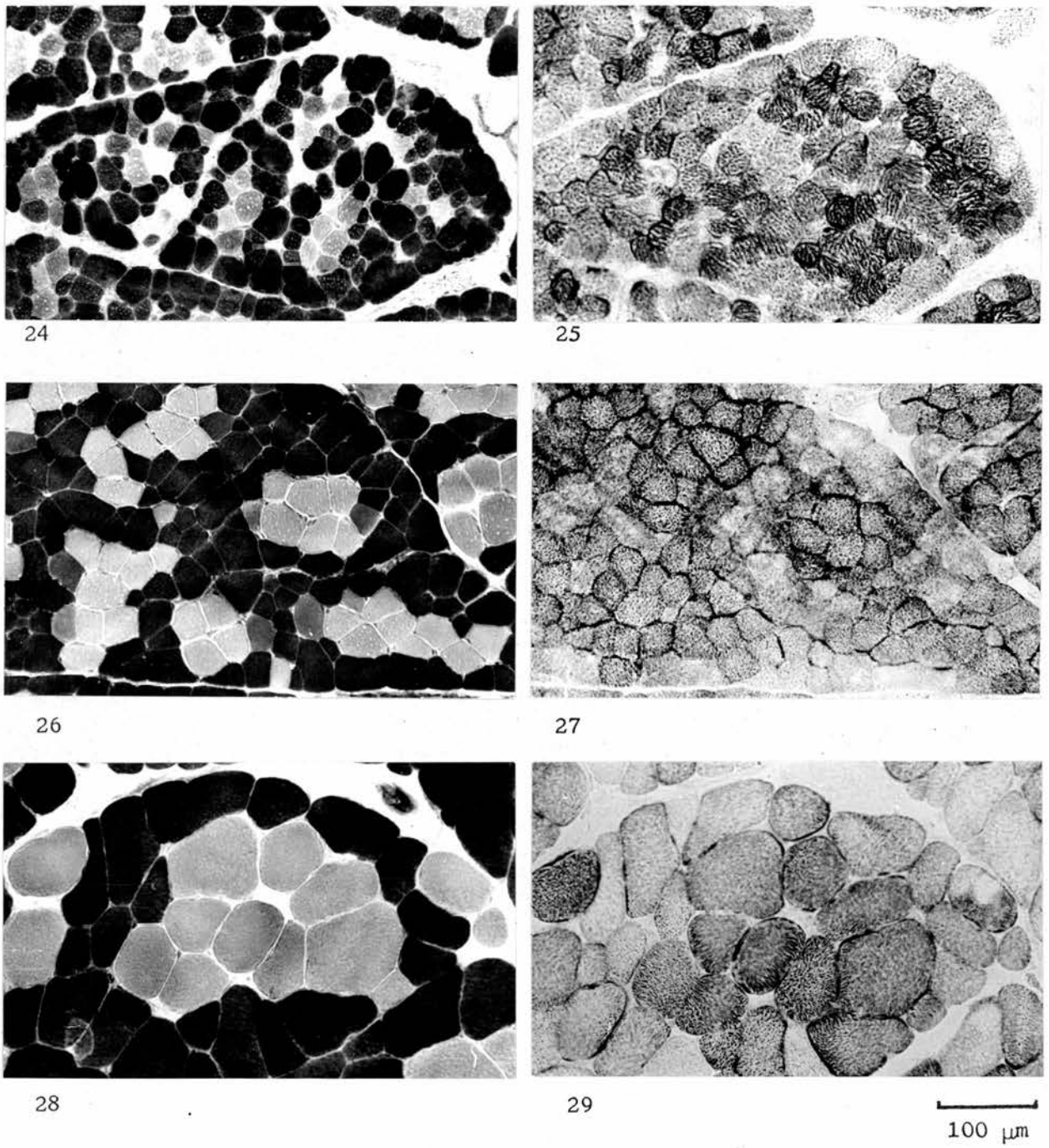
Table 5. Longissimus and diaphragm; Series 1 and 2
Fibres low in myosin ATPase (A1), SDHase (S1) and GPase (P1) as a percentage of total fibres sampled

Pig No.	Live-weight (kg)	Percentage of Fibres					
		Longissimus			Diaphragm		
		S1	P1	A1	S1	P1	A1
1	1.27	*	*	2.0	*	*	10.4
2	3.69	63.8	5.8	4.3	*	*	18.5
3	3.72	62.7	5.6	4.0	42.3	36.6	20.7
4	3.98	63.3	4.0	3.2	*	24.6	20.0
5	4.16	62.1	2.9	2.9	38.1	38.1	24.3
6	7.33	64.1	8.2	5.5	32.6	37.0	26.6
7	7.69	62.2	7.1	5.3	41.8	33.1	25.7
8	8.11	58.5	16.1	9.2	23.8	12.2	22.5
9	9.46	61.2	11.6	5.5	38.4	23.5	22.7
10	13.0	58.4	13.5	3.9	20.5	31.6	29.9
11	13.5	61.0	7.5	7.7	29.2	47.7	36.9
12	15.0	57.4	15.3	9.3	24.7	31.6	28.7
13	25.0	56.5	-	4.1	34.3	41.1	39.9
14	27.8	63.4	-	8.9	22.1	35.1	36.5
15	28.9	74.5	14.3	7.3	32.9	28.1	36.2
16	57.4	82.4	14.5	3.3	38.8	30.0	33.9
17	59.0	55.6	14.7	13.6	31.0	34.5	36.2
18	59.6	72.6	14.3	9.3	28.4	34.1	36.9
Series 2 (N = 16)							
mean	93.0	64.7	28.6	18.6	18.3	39.9	36.5
SD	2.8	4.8	11.6	4.5	7.3	8.1	5.9

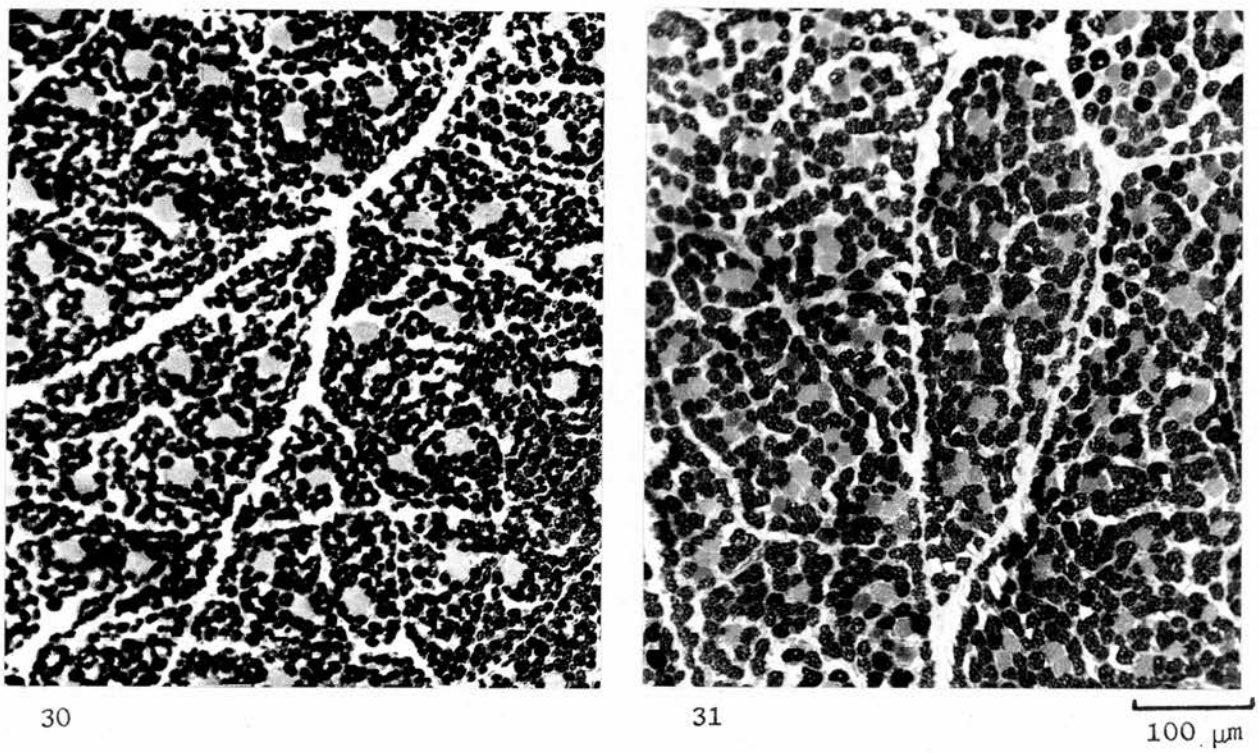
* Indicates that fibres were insufficiently differentiated for quantitation by the histochemical reactions.



Figs. 18-23. Transverse serial fresh frozen sections of m. longissimus of the Large White pig at the same three stages of growth shown in Fig. 17; liveweights 4·0 kg (upper), 13 kg (middle) and 98 kg (lower). Sections are stained to demonstrate the activity of myosin ATPase (left) and SDHase (right).



Figs. 24-29. Transverse serial fresh frozen sections of the diaphragm of the Large White pig at the same three stages of growth shown in Fig. 17; liveweights 4·0 kg (upper), 13 kg (middle) and 98 kg (lower). Sections are stained to demonstrate the activity of myosin ATPase (left) and SDHase (right).



Figs. 30, 31. Transverse fresh frozen sections of longissimus (Fig. 30) and diaphragm (Fig. 31) of a Large White pig, liveweight 1·3 kg, age 2 days. Myosin ATPase.

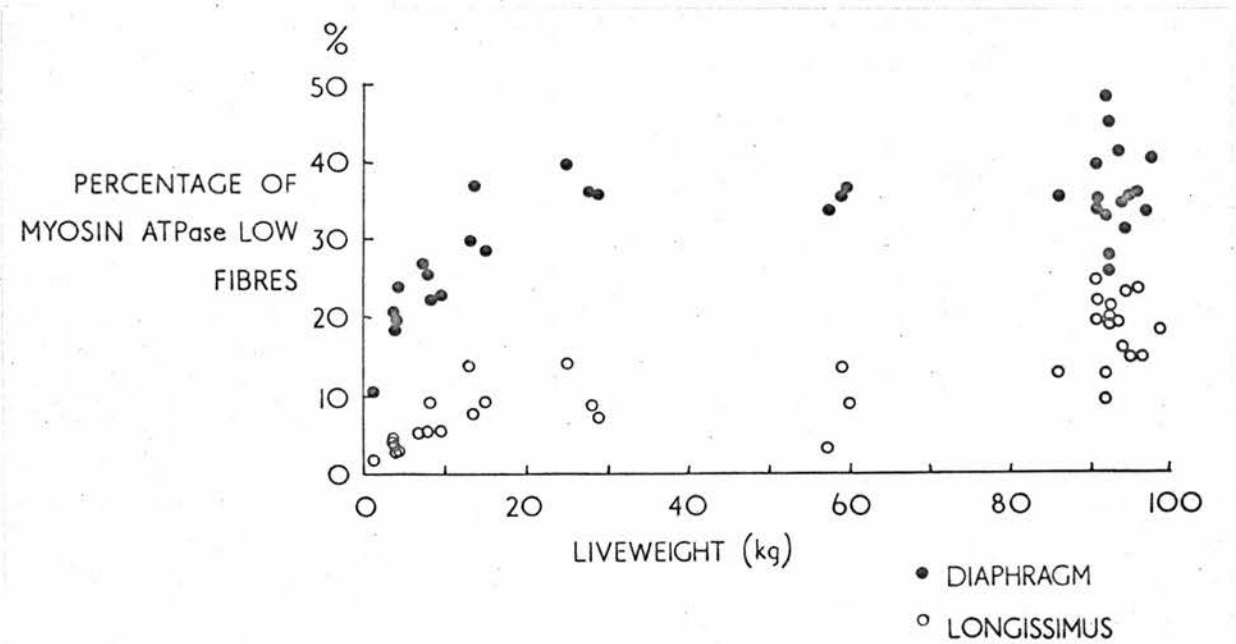


Fig. 32. Longissimus and diaphragm of 34 Large White pigs (Series 1 and 2). Growth changes in the proportion of myosin ATPase low fibres.

proportion of GPase low fibres than myosin ATPase low fibres over this period (Table 5). The diaphragms of the older pigs of Series 1, and longissimus muscles of all pigs subsequent to the early differentiation process, are principally composed of the three main fibre types previously described for the pigs of Series 2.

2.3.2.5 Changes in the 'myosin ATPase low bundles' in longissimus (Table 6)

The pigs of Series 1 are divided into four groups, A-D. Group A consists of only one pig of 1·3 kg liveweight. Groups B-D comprise pigs of liveweights ranging from 3·0 to 7·5 kg, 7·5 to 15 kg and 15 to 60 kg respectively. Group E includes all the pigs of Series 2. Although the estimate of the total number of myosin ATPase low bundles in a complete transverse section of longissimus is significantly lower in group B than in group C, the estimate of the number of bundles does not vary significantly between the other groups, suggesting that the number of myosin ATPase low bundles in longissimus does not change during

Table 6. **Growth changes in the myosin ATPase low bundles of m. longissimus**

Group	Range of liveweights (kg)	No. of pigs	Estimate of No. of bundles in T.S. of muscle ($\times 10^4$)		No. of myosin ATPase low fibres per bundle	
			mean	SD	mean	SD
A	1.3	1	2.35	-	1.0	-
B	3.0 - 7.5	5	1.64	0.42	1.20	0.20
C	7.5 - 15	6	2.60	0.73	1.99	0.40
D	15 - 60	6	2.22	0.67	2.21	0.61
E	85 - 97	16	2.70	0.42	3.24	0.50

the period of growth studied. There is, however, a steady increase in the mean number of myosin ATPase low fibres per bundle between all stages of growth; the difference is significant between groups B and C, and between groups D and E. This change is also seen by comparing Figs. 18 and 30 with Figs. 20 and 22.

2.3.2.6 Occurrence of 'transitional' fibres (Table 7; Figs. 33-36)

Especially in pigs between birth and 15 kg liveweight, fibres with an intermediate reaction for myosin ATPase are seen in both longissimus and diaphragm. These fibres are always adjacent to myosin ATPase low bundles. A large number of these fibres is seen in a sample of diaphragm from a 13 kg, 56 day old pig (Figs. 33-35). Fibre profiles were determined for this sample, and areas were measured by the paper weighing method. Details of the fibre types are shown in Table 7. In this classification, myosin ATPase high fibres are not differentiated by the SDHase reaction. Myosin ATPase high, intermediate and low fibres are classified as high, intermediate and low by the GPase reaction; elsewhere in this study GPase intermediate fibres are classified as GPase low (see paragraph 2.2.4).

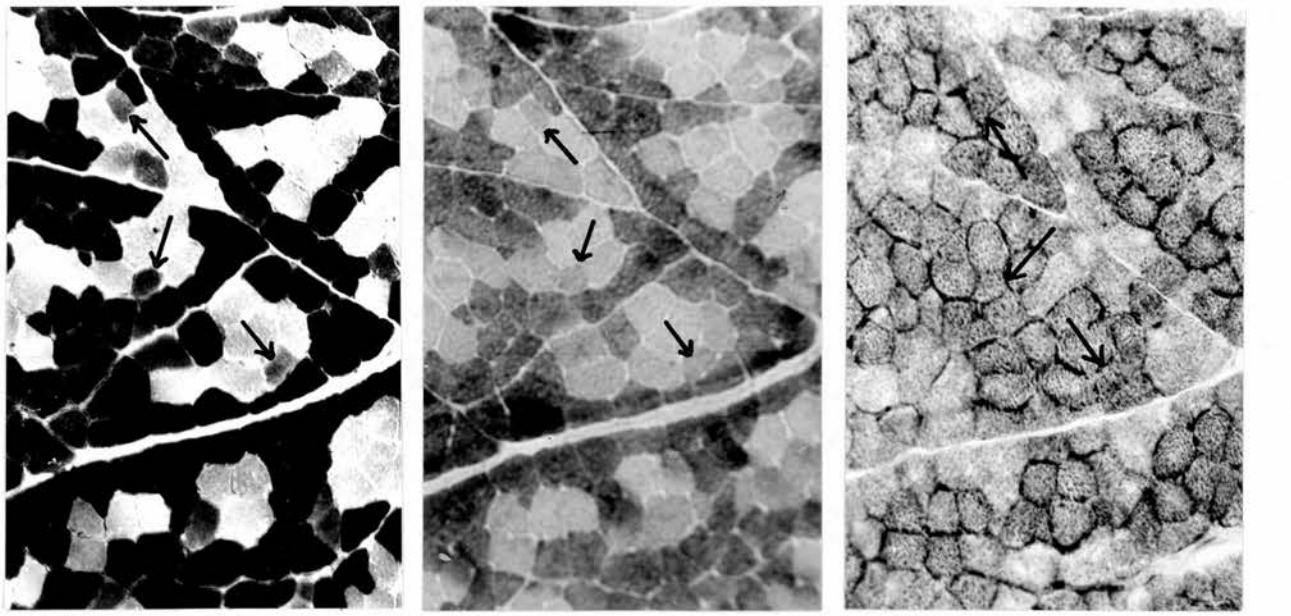
The following observations may be listed:

(i) 'Transitional' fibres have an SDHase activity as high as that of myosin ATPase low fibres, but a smaller mean TSA.

(ii) Large myosin ATPase low fibres have a lower GPase reaction.

(iii) The gradation in mean TSA and in the myosin ATPase and GPase reactions between these fibre types suggests a transition from one type to another in the order shown in Table 7.

(iv) The 'transitional' fibres appear to originate from those fibres of the



Figs. 33-35. Transverse serial fresh frozen sections of the diaphragm of a Large White pig, liveweight 13 kg, age 56 days, stained to demonstrate the activity of myosin ATPase (Fig. 33), GPase (Fig. 34) and SDHase (Fig. 35). Arrows indicate examples of 'transitional' fibres.

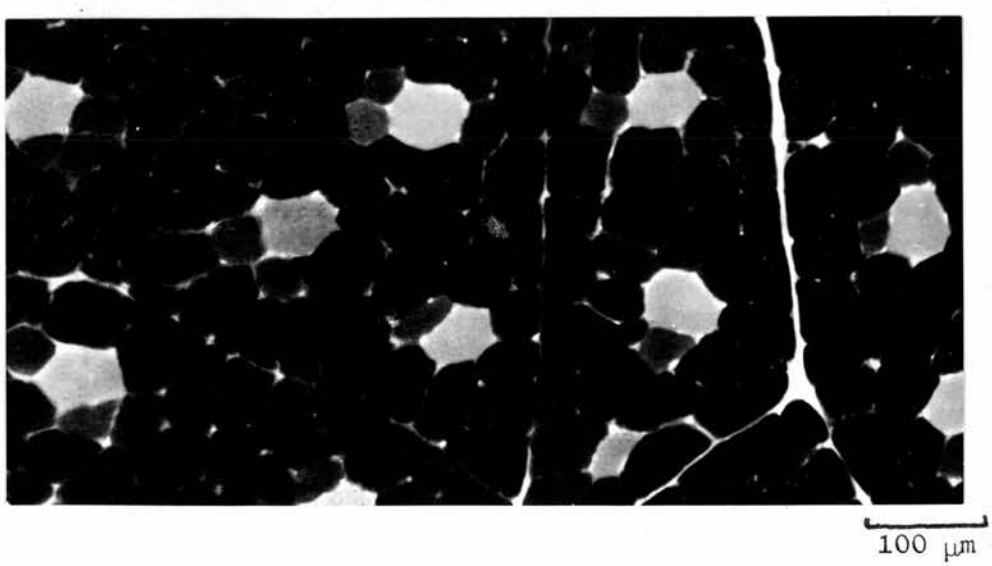


Fig. 36. Transverse fresh frozen section of m. longissimus of a Pietrain pig, liveweight 14 kg, age 61 days, stained to demonstrate the activity of myosin ATPase. Fibres with an intermediate reaction for this enzyme occur adjacent to myosin ATPase low fibres.

Table 7. Histochemical profiles of "transitional" and other fibre types in the diaphragm of a Large White pig, liveweight 13 kg.

Fibre type	Percentage of fibres (N = 1,232)	Mean TSA (μm^2)	Percentage area of muscle (Area sampled = 1.2 mm^2)
myosin ATPase high SDHase high or low GPase high or intermediate	64.6	957	63.3
myosin ATPase intermediate SDHase high GPase intermediate ("transitional" fibre)	6.3	773	5.0
myosin ATPase low SDHase high GPase intermediate	10.6	934	10.2
myosin ATPase low SDHase high GPase low	18.4	1142	21.5

first type that are myosin ATPase and SDHase high, are GPase intermediate, have a low mean TSA, and are adjacent to the myosin ATPase low bundles.

2.3.2.7 Changes in the TSA of longissimus occupied by myosin ATPase low fibres (Fig. 37)

The relationship between liveweight and both the complete TSA of longissimus, and the TSA occupied by myosin ATPase low fibres, is represented in Fig. 37 as a double logarithmic regression for the pigs of both series. The slopes of the two regression lines are significantly different ($P < 0{\cdot}001$) from one another. The regression coefficient for the TSA of the whole muscle is not significantly different ($P > 0{\cdot}05$) from 0·67, and the regression coefficient for the TSA occupied by myosin ATPase low fibres is not significantly different ($P > 0{\cdot}05$) from 1.

These results support the hypotheses that the TSA of a muscle, which is growing proportionately to the rest of the body, varies as the 2/3 power of the body weight; and that, in such a muscle, the TSA occupied by myosin ATPase low fibres bears a direct proportion to body weight.

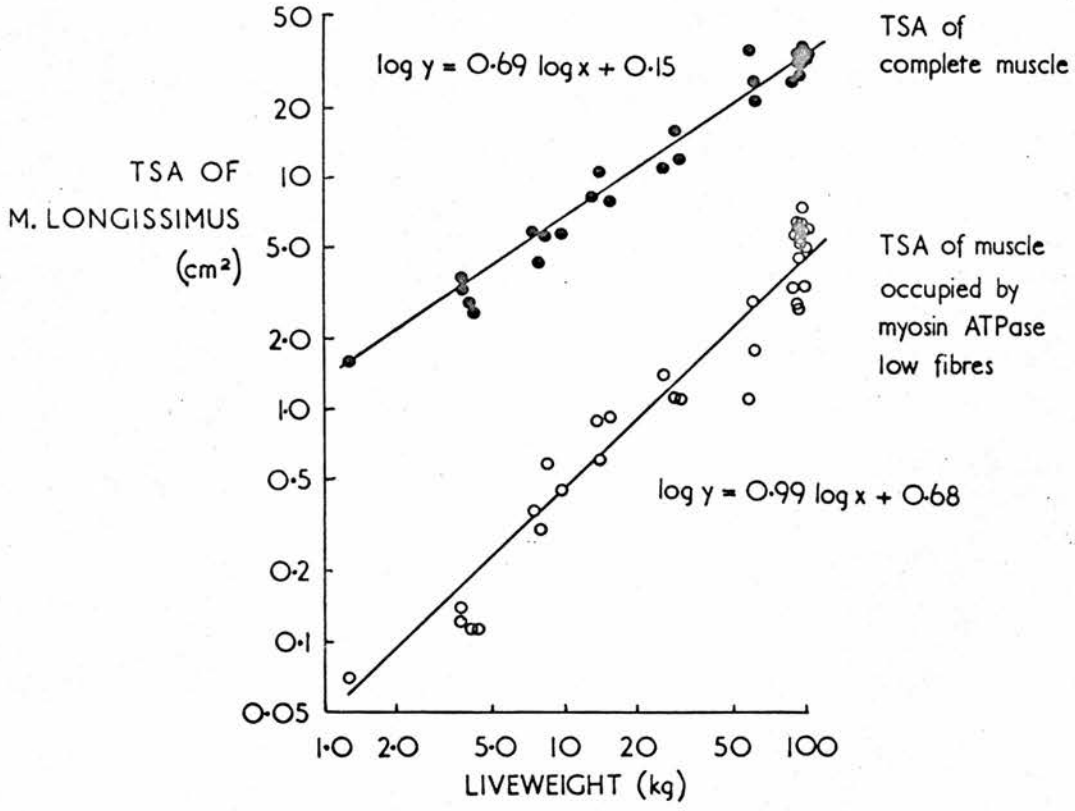


Fig. 37. Growth changes in m. longissimus of 34 Large White pigs at the thoracolumbar junction (Series 1 and 2). Total transverse sectional area compared with the area occupied by fibres low in myosin ATPase activity.

2.4 DISCUSSION

2.4.1 Significance of the histochemical reactions used

Succinate dehydrogenase

Patterns of mitochondria, demonstrated by classical methods (Nachlas, et al., 1957; Scarpelli & Pearse, 1958; Novikoff, Shin & Drucker, 1961) or by electron microscopy in skeletal muscle (Padykula & Gauthier, 1963; Ogata, 1964; Pieper, Feustel & Hübner, 1969) and kidney (Novikoff et al., 1961), are in each case shown to follow the diformazan deposition caused by SDHase activity. Brooke & Engel (1966) provide evidence that nitro BT is selectively adsorbed on to mitochondria and sarcoplasmic reticulum of striated muscle fibres. Since, however, SDHase is believed to be entirely intramitochondrial (Roodyn, 1967), this should enhance the histochemical localisation of this enzyme. A limited extent of diformazan deposition away from sites of SDHase activity, such as lipid droplets (Hitzeman, 1963), should have little effect on the comparison between individual fibres, but the report of a heterogeneous all-or-none deposition in individual mitochondria (Seligman, Ueno, Morizono, Wasserkrug, Katzoff & Hanker, 1967) could have more serious implications. It is possible that the SDHase activity of mitochondria from different fibres may vary (Blanchaer, 1964), but the density of diformazan deposited histochemically in a particular fibre after incubation for as long as 20 minutes should depend primarily on mitochondrial density, rather than on the actual level of SDHase activity.

Paul & Sperling (1952) demonstrate a direct relationship between estimates of mitochondrial density, determined by phase microscopy of blendorised tissue, and the oxidative capacity of a variety of muscles from different species. This relationship is supported by observations on the effect of severe exercise

on limb muscles, which can produce a two-fold increase in the capacity of muscle to oxidise pyruvate (Holloszy, 1967), accompanied by a concomitant increase in mitochondrial density as seen electron microscopically (Gollnick & King, 1969). Similar findings are reported by Kraus, Kirsten & Wolff (1969).

The assumption that the histochemical SDHase reaction indicates the capacity of an individual fibre for aerobic metabolism appears reasonable, although it lacks direct proof.

Phosphorylase

Takeuchi & Kuriaki (1955) show that their method is specific for the enzyme catalysing the successive phosphorylation of the terminal glucose units of the glycogen chain, with the production of glucose-1-phosphate. The method uses the reversibility of this reaction to synthesise a polyglucose staining blue with iodine, that is distinct from native glycogen, both by iodine staining and by electron microscopic appearance (Takeuchi & Sasaki, 1968). Differences in the colour of iodine staining are attributed to the progressive increases in chain length during synthesis of the glucose polymer, blue indicating chains of over 30 glucose units, and red indicating chains of 7-13 glucose units (Swanson, 1948). Iodine colours are used in this study to indicate different levels of phosphorylase activity in individual fibres.

Using muscles from a wide variety of vertebrates and invertebrates, Crabtree & Newsholme (1972) show biochemically that GPase activity is closely related to the capacity of a muscle for anaerobic metabolism. Provided that sufficient glycogen is present (Meijer, 1968a), it is accepted that the GPase activity demonstrated histochemically in an individual fibre is a measure of the rate at which the fibre derives energy for contraction anaerobically.

Myosin ATPase

Padykula & Herman (1955) and Padykula & Gauthier (1963) provide evidence that their histochemical technique is specific for myosin ATPase. This is given strong support by the work of Guth & Samaha (1969), who compare the effects of pre-incubation at pH values of 10·4 and 4·3 on the ATPase activity of both actomyosin extracted from fast and slow muscles of the cat, and individual fibres of these muscles examined histochemically. Their study also provides evidence that fibres shown histochemically to be ATPase high are fast contracting, and that ATPase low fibres are slow contracting. This concept is supported by the work of Burke, Levine, Zajac, Tsairis & Engel (1971), who correlate the twitch contraction time of motor units in the cat gastrocnemius determined by intracellular stimulation of single motoneurons, with the histochemical profiles of component fibres of the related motor units, identified by glycogen depletion following repetitive stimulation. Biochemical evidence is provided by the finding that the activity of myosin ATPase is directly proportional to the intrinsic speed of shortening both of normal muscles of widely varying speeds of contraction (Bárány, 1967), and of muscles in which the speed of contraction has been altered by cross-innervation (Bárány & Close, 1971). The distinct difference between the histochemical reactions of fast and slow contracting fibres is possibly related to the molecular difference between the myosin of fast and slow muscles demonstrated by Samaha, Guth & Albers (1970).

This evidence appears to justify the designation of ATPase high mammalian extrafusal fibres as fast-twitch, and ATPase low fibres as slow-twitch fibres.

2.4.2 Classification of fibre types

The interpretation of histochemical fibre types should relate the reactions directly to the physiological and metabolic characteristics of each fibre. The evidence given above suggests that the profile obtained by determining the SDHase, phosphorylase and myosin ATPase reactions will classify an individual fibre by its capacity for aerobic and anaerobic metabolism, and by its intrinsic speed of contraction. Accepting that a fibre classified as 'anaerobic' or 'aerobic' will usually have a low level of the other type of metabolism, for both fast-and slow-twitch fibres there are three theoretical possibilities for their metabolism; aerobic, combined aerobic and anaerobic, and anaerobic. Five of these six possible fibre types are found in significant proportions in the diaphragms of nine different mammals (Davies & Gunn, 1972). The present study shows that the number of fibre types occurring in proportions above 5% of the total fibre population, and therefore contributing significantly to the function of the porcine longissimus and diaphragm, is four in pigs aged from 10 to 56 days, and only three in older pigs.

The Ah,Sh,Ph fibre constitutes 41·0% of the fibre population of the diaphragm of the pig, and 13·9% of the fibres in m. longissimus. Although the histochemical methods do not indicate what the absolute levels of activity of SDHase and GPase might be, the presence of significant proportions of this fibre type suggests that the relationship between aerobic and anaerobic metabolism in muscle fibres is not necessarily a 'reciprocal' one, as claimed for fibres of rat, human (Dubowitz & Pearse, 1960a, b) and cat (Jinnai, 1960) muscles, and supported by Engel (1962, 1965, 1970) and Suchenwirth & Bundschu (1970) for human muscles; Nishiyama (1966) for respiratory muscles of the rat and cat;

Kugelberg & Edström (1968) for rat crural muscles; and by Jasmin, Bokdawala & Desrosiers (1971) for crural muscles of the hamster. However, there is no reason to suppose that the two systems of energy production in muscle outlined in Fig. 38 should not both contribute appreciably to ATP production in a muscle fibre adapted to rapid and frequent contraction. Other studies support the present histochemical evidence for this; fibres high in SDHase and moderate to high in GPase activity are demonstrated in rat crural muscles (Romanul, 1964), in rat femoral muscles and m. soleus of monkey and rat (Bocek & Beatty, 1966),

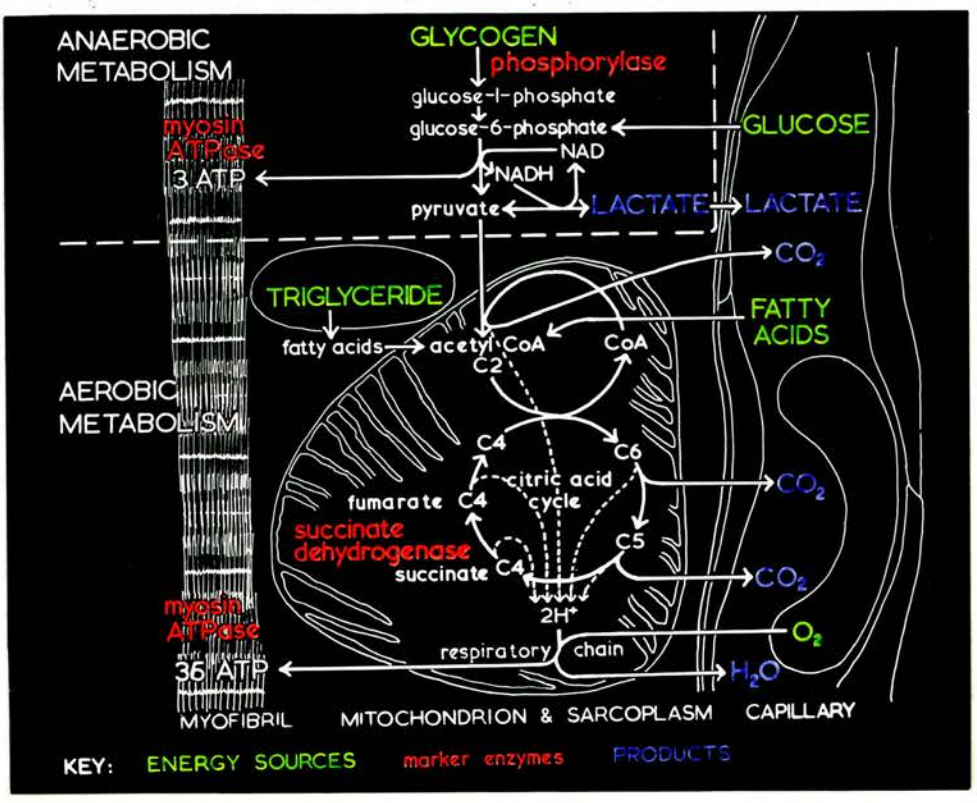


Fig. 38. Outline of aerobic and anerobic metabolism in muscle. The dotted line separates a system of energy production (top left) for which a blood supply, and therefore an extrinsic energy source and a means of disposal of unmetabolisable products, is unnecessary. An oxygen supply is necessary for energy production from the remainder of the system.

in the thyroarytenoid and cricothyroid muscles of the rabbit (Hall-Craggs, 1968), in guinea-pig crural muscles (Edgerton & Simpson, 1969), in cat crural muscles (Prewitt & Salafsky, 1970), in the gastrocnemius and soleus muscles of the mouse and triceps brachii and rectus abdominus muscles of the pig and ox (Ashmore & Doerr, 1971), and in the diaphragm of eight mammalian species (Davies & Gunn, 1972). In addition, Gillespie, Simpson & Edgerton (1970), find greater stores of glycogen in the 'red' region of m. vastus lateralis of the guinea-pig, composed of 77% SDHase high fibres, than in the 'white' region, composed of 29% SDHase high fibres.

Edgerton & Simpson (1969) review the various classifications that have been used since 1962 for histochemical fibre types in muscle. They favour the descriptive terms 'red', 'intermediate' and 'white' in preference to letters or numbers. Fibres low in myosin ATPase activity are described as 'intermediate' in SDHase activity by Stein & Padykula (1962), Edgerton & Simpson (1969) and Jasmin, Bokdawala & Desrosiers (1971) in their studies of crural muscles of rat, guinea-pig and rat, and hamster respectively. However, the present results, and those of Ashmore & Doerr (1971) for limb muscles of the pig and ox, and Burke et al. (1971) for the cat gastrocnemius, show that this type of fibre frequently has SDHase activity equal to or greater than surrounding myosin ATPase high fibres. The term 'intermediate' has, therefore, no general significance.

A classification of fibre types has little meaning unless histochemical profiles of large numbers of fibres are first determined, enabling quantification of both the proportions of types, and the variance of these proportions between samples. The actual classification made will depend on the histochemical methods used and the visual interpretation of the reactions in each fibre.

The methods used in the present study are justified by their simplicity and their significance. The value of the classification used can best be tested by comparing the measurements on fibre types within a muscle with the physiological and biochemical characteristics of the muscle, and by using these measurements to test hypotheses interpreting experimental and developmental changes.

2.4.3 Histochemical fibre types in porcine muscle

The grouping of histochemical fibre types into bundles within the fasciculi of porcine muscle is demonstrated by methods for reduced nicotinamide adenine dinucleotide tetrazolium reductase ($NADH_2$-TR) and GPase (Moody & Cassens, 1968) lipids (Todorov & Petrov, 1969), myoglobin and $NADH_2$-TR (Morita, Cassens & Briskey, 1969), SDHase, cytochrome oxidase and lipids (Sair, Lister, Moody, Cassens, Hoekstra & Briskey, 1970) and $NADH_2$-TR, GPase and myosin ATPase (Cooper, Cassens, Kastenschmidt & Briskey, 1970). Although the distribution of fibre types is probably not random in any species, as shown by James (1971a, b; 1972) for rabbit and guinea-pig muscles and by Jennekens, Tomlinson & Walton (1971b) for human muscles, the muscles of the pig so far examined exhibit a much more orderly arrangement than those of any other species described. No reports are available on the muscle histochemistry of other species within the suborder Suiformes.

Moody & Cassens (1968) describe the histochemistry of longissimus and trapezius of the Chester White pig at 90 kg liveweight by the use of reactions for $NADH_2$-TR and GPase. They find that the fibre population of longissimus is composed of 30·5% (SD = 5·0) aerobic fibres and 17·0% (SD = 4·3) GPase low fibres. These findings are similar to the present estimations of 35·3% (SD = 4·8) of aerobic fibres and 28·6% (SD = 11·6) of GPase low fibres (Table 5).

These workers do not, however, establish histochemical profiles of individual fibres, and so cannot assess the GPase activity of individual aerobic fibres; hence their conclusion that the activity of these two enzymes is reciprocal is questionable, and is not supported by the present observations. The 'intermediate' (fast-twitch, combined aerobic and anaerobic) fibre of porcine muscle described by Moody & Cassens (1968), by Merkel (1971) for m. gluteus medius and m. rectus femoris and by Sair, Kastenschmidt, Cassens & Briskey (1972) for m. longissimus, is not equivalent to the slow-twitch aerobic fibre described as having 'intermediate' aerobic capacity in crural muscles of the rat and guinea-pig by Edgerton & Simpson (1969). The term 'intermediate' is misleading in either context.

Meijer (1968a) shows that the histochemical demonstration of GPase depends on the presence of tissue glycogen. Therefore, when complete antemortem or postmortem glycolysis has occurred in a muscle fibre, GPase cannot be demonstrated by the method used in this study. Regions with very few GPase high fibres are seen in some samples from commercially slaughtered pigs. Their focal nature suggests that they are due to postmortem glycolysis, since this phenomenon can occur to a different extent in closely adjacent regions of the same muscle (Lawrie, Gatherum & Hale, 1958). The existence of GPase activity in these affected fibres could be tested by comparing them with serial sections incubated in a medium containing dextran (Meijer, 1968b).

2.4.4 Relation between size, metabolism and rate of energy conversion of muscle fibres

The time taken for oxygen to diffuse into the centre of a fibre is proportional to its transverse sectional area (Hill, 1965). Hence, the TSA of a fibre would be expected to be influenced both by its dependence on aerobic metabolism and by the rate of energy conversion within the fibre.

An inverse relationship between fibre TSA and aerobic capacity is well established. In the biceps brachii of mice, predominantly aerobic fibres are narrower (Goldspink, 1969) and, in the present study, myosin ATPase high fibres in the longissimus and diaphragm have smaller TSAs if the SDHase activity is high. Fibres converting energy at the same rate, as determined by their myosin ATPase activity, are more slender and have more adjacent capillaries (Romanul, 1965; Cooper, Cassens & Briskey, 1969) if they depend on oxygen and blood-borne nutrients for their energy source.

The influence of rate of energy conversion on the TSA of a fibre has not received the same attention. In the diaphragm of the pig the myosin ATPase low fibres have the densest reaction for SDHase and a greater mean TSA than the surrounding myosin ATPase high fibres, which react for both aerobic and anaerobic enzymes. Thus, it appears that when the energy demands are low, a large diameter fibre can obtain sufficient fuel for a predominantly aerobic metabolism.

Only fast-twitch anaerobic fibres are significantly different in TSA between muscles as diverse in function as the diaphragm and longissimus of the pig. Jennekens, Tomlinson & Walton (1971a) show that slow-twitch aerobic fibres are relatively larger in the rectus femoris and gastrocnemius than in the deltoid and biceps brachii muscles of man. Engel, Brooke & Nelson (1966) demonstrate the relative susceptibility to atrophy of slow-twitch fibres following tenotomy and fast-twitch fibres following experimental denervation. The extent to which the differences in mean fibre TSA between muscles of mature animals, and between individuals of the same species, are due to the relative hypertrophy of different types of fibres is relevant to the effect of breed, sex, nutrition, exercise and disease on muscle development.

2.4.5 Neonatal fibre type differentiation

Changes in the histochemical fibre types of mammalian muscles are observed during the immediate post-natal period. The findings of Wirsen & Larsson (1964), Dubowitz (1965), Beatty, Basinger & Bocek (1967), Dorn (1969), Ommer (1971) and Ashmore, Tompkins & Doerr (1972) demonstrate a variation in the time of onset of differentiation of an anerobic fibre type in muscles of the mouse, rat, hamster, guinea-pig, rabbit, cat, rhesus monkey, pig, sheep, man and ox. Dubowitz (1965) considers that this variation depends on the relative maturity of the species at birth. Nyström (1968) observes topographical differences in the onset of differentiation in individual muscles of the cat. Thus, fibres with low activity of SDHase have already appeared in forelimb muscles, intercostal muscles and diaphragm at birth, at a time when hindlimb muscles retain a uniformly high reaction. Cooper, Cassens, Kastenschmidt & Briskey (1970) state that fibre types in longissimus of the neonatal pig are undifferentiated, although their published photographs of serial sections from a one-day-old pig appear to show several fibres of large TSA that also differ from surrounding fibres by their low activity of myosin ATPase and GPase. Their statement is contrary to the findings both of the present investigation and to that of Nyström (1968) that the gastrocnemius and soleus of the relatively immature neonatal kitten is differentiated with respect to myosin ATPase and GPase.

Wohlfart (1937), who studied fixed and stained preparations of a wide variety of human fetal and neonatal muscles, describes the presence of 'b' fibres that differ from the surrounding 'a' fibres because of their relatively large size. They occur singly, but two or three can be seen in the same fasciculus. They form between 0·5 and 2·5% of all fibres at birth. Fenichel (1963) shows that

Wohlfart's 'b' fibre is low in myosin ATPase activity, and there is little doubt that this fibre type is the myosin ATPase low fibre seen in neonatal porcine muscles.

The above studies are concerned with changes due to the different functional requirements of muscle in a prenatal and a postnatal environment, rather than with the adaption of muscle to meet the mechanical and metabolic demands of increased body size.

2.4.6 Mechanical adaption of muscles to increasing body size

Welcker & Brandt (1903) suggest that larger species of animals need a higher proportion of muscle and bone than smaller species, in order to support and move their bodies with structures whose strength is proportional only to their TSA. However, the data they record for the mouse, bat, hedgehog, guinea pig, monkey, ox and elephant do not support their hypothesis. Jackson & Lowry (1912) review other findings and conclude that comparatively small animals, such as the rabbit and cat, have the highest proportions of muscle, although these workers demonstrate that growth in the rat results in an increase in the weight of muscle as a proportion of body weight from 23% at one week to 45% at one year of age. In the pig, the proportion of muscle decreases slightly as liveweight increases from 23 kg to 118 kg (Cuthbertson & Pomeroy, 1962; Stant, Martin, Judge & Harrington, 1968; Richmond & Berg, 1971a).

Because an increase in body size is not associated with an increase in the proportion of muscle, it is to be expected that the contractile properties of postural muscles must adapt to the changing demands placed upon them. For animals maintaining the same dimensional proportions, Hill (1950) predicted that

the intrinsic speed of contraction of muscles would decrease with increasing body size. No physiological studies have been made specifically to test Hill's hypothesis. Both fast- and slow-twitch muscles of neonatal kittens (Buller, Eccles & Eccles, 1960b; Buller & Lewis, 1965; Mann & Salafsky, 1970) and rats (Close, 1964) are initially slow contracting; the subsequent differentiation of contraction speed may be associated with the development of normal muscle usage in animals born in an immature state. These workers also show that the time to peak tension of soleus of both the rat and cat increases between 40 and 100 days of age; the rat soleus from 28·5 $\pm$ 2·3 ms at 35 days to 36·0 $\pm$ 2·3 ms at 100 days (Close, 1964), and the cat soleus from 59 ms at 42 days to 75 ms at 126 days (Mann & Salafsky, 1970). They do not, however, comment on the significance of this later change.

The developing soleus muscle of the rat, guinea-pig and cat was studied histochemically by Karpati & Engel (1967b). Their findings for the cat were confirmed by Nyström (1968). At birth, the soleus of the guinea-pig has approximately equal numbers of myosin ATPase high and low fibres. The rat soleus, undifferentiated at birth, resembles the cat and guinea-pig soleus by 10 days. The soleus of the adult cat and guinea-pig is almost entirely composed of myosin ATPase low fibres; about 90% of the fibres in the adult rat soleus are myosin ATPase low. A similar increase in the proportion of myosin ATPase low fibres occurs with increasing body size in the pectineus muscle of the dog (Cardinet, Wallace, Fedde, Guffy & Bardens, 1969), and in an inter-species comparison of m. semitendinosus (Davies & Gunn, 1971) and the diaphragm (Davies & Gunn, 1972). However, the observations of Cooper, Cassens, Kastenschmidt & Briskey (1970) on fibre types in m. longissimus of the pig from

birth to 90 kg liveweight contradict the above findings, and those of the present study. They report a decrease in the proportion of the TSA of the muscle occupied by myosin ATPase low fibres with increasing body size, and their illustrations do not show an increase in the number of fibres per myosin ATPase low bundle.

Edgerton & Simpson (1969) suggest that the proportion of myosin ATPase low fibres demonstrated histochemically in a muscle is related directly to the contraction time. This is confirmed by Barnard, Edgerton, Furukawa & Peter (1971), Cardinet, Fedde & Tunell (1972) and Davies & Gunn (1972). The histochemical changes seen in the muscles of growing animals show that changes in the contractile properties of muscle depend on body size. The direct relationship demonstrated between body weight and the TSA of a muscle occupied by myosin ATPase low fibres (Fig. 17) supports the concept that the histochemical change occurs in response to a demand on a muscle for prolonged isometric contraction, directly proportional to the weight it must support.

This response has not been studied biochemically. Trayer & Perry (1966) report that the ATPase activity of myosin extracted from muscles of the fetal rat, guinea-pig and rabbit is lower than that of adult myosin. Guth & Samaha (1972) confirm this observation on rabbit muscle. They also claim that all fibres show an intense histochemical reaction for myosin ATPase in rabbit limb muscles at birth, although large fibres with a less intense reaction are apparent in their illustration of a rat hind limb muscle. Since the histochemical method demonstrates a difference between fibres in the alkali stability of myosin ATPase (Guth & Samaha, 1969) rather than the overall activity of this enzyme, the difference between the biochemical and histochemical findings does not

necessarily belittle the ability of the histochemical method to differentiate fibre types on a functional basis. In any case, this apparent biochemical difference between fetal and adult myosin may not be directly associated with its ATPase activity (Dow & Stracher, 1971).

2.4.7 Metabolic adaption of muscles to increasing body size

Increase in body size imposes restrictions on the ability of the respiratory and circulatory systems to supply oxygen to muscle. The total surface area of the lung alveoli is shown by Tenney & Remmers (1963) to be proportional to the rate of oxygen consumption of the whole body, or the 3/4 power of body weight (Kleiber, 1947), of different mammalian species. This area is also proportional to the surface area of the human body during growth (Dunnill, 1962), rather than directly to body weight. Similarly, the TSA of the aorta, and hence the ability of the heart to supply oxygenated blood to the body tissues, cannot maintain a direct proportionality to the body weight (Hill, 1950). Thus, although small vital organs and postural muscles demanding relatively low energy conversion rates for isometric contraction can retain a purely oxidative metabolism during growth, the muscles which a large animal uses mainly for brief periods of intensive propulsive force must adapt to obtain energy anaerobically.

Studies that might support or refute this hypothesis are difficult to interpret. The in vitro respiration experiments of Bertalanffy & Pirozynsky (1953) on the diaphragm of the mouse, Bertalanffy & Estwick (1953) on the diaphragm and leg muscles of the mouse and rat, Latzkovits & Domonkos (1965) on longissimus, abdominal muscles and soleus of the rabbit, and van Den Hende, Muylle, Oyaert & de Roose (1971) on longissimus of the pig do not examine the complete metabolic capability of muscle in the intact animal. The studies by Goldspink (1962) on

the activity of SDHase in the biceps brachii of the mouse, by Markert & Ursprung (1962) on the changing proportions of lactate dehydrogenase isoenzymes during growth of the mouse diaphragm and 'skeletal muscle', by Kendrick-Jones & Perry (1967) on enzymes of adenine nucleotide metabolism in diaphragm and leg muscles of the rabbit, and by Bocek, Basinger & Beatty (1969) on enzymes concerned with glycogen synthesis and breakdown in limb muscles of the rhesus monkey, are not dissociated from the adaptive changes inherent in the transition from a maternal dependent to an independent environment. Cooper, Cassens, Kastenschmidt & Briskey (1971) studied lactate dehydrogenase (LDHase) and GPase in the developing longissimus and trapezius muscles of Poland China and Yorkshire pigs from birth to 90 kg liveweight. The changes occurring in neonatal pigs are also difficult to interpret, but from 8 weeks of age (presumably about 5 kg liveweight) to 90 kg, their results appear relevant to the present study. Over this weight range, both muscles show the same trend; the total LDHase activity increases, and the proportion of this activity (LDHase isoenzyme 5) favouring lactate production from pyruvate increases, while the total GPase activity decreases. The activity of LDHase and GPase is lower, and the proportion of aerobic fibres is higher (Moody & Cassens, 1968), in trapezius. These results are consistent with the concept that with increasing body size, the pyruvate produced from glycolysis in both muscles is converted increasingly to lactate, rather than passing to the respiratory chain via the citric acid cycle. Lactate production is greatest in the propulsive longissimus muscle.

Although it is possible by histochemical methods to estimate the relative capacity of adjacent fibres for a particular metabolic process, the proportion of fibres with low activity of a particular enzyme does not necessarily indicate

the overall enzymic activity of different muscle samples. Therefore, the results of the present study suggesting that subsequent to an initial neonatal differentiation, the proportion of SDHase low fibres of both longissimus and diaphragm does not increase with growth (Table 5), cannot be considered as evidence that the aerobic capacity of the muscles does not change. Nevertheless, an increase in the proportion of SDHase low fibres with growth has been reported for the guinea-pig plantaris (Faulkner, Maxwell, Brook & Lieberman, 1971) and diaphragm (Lieberman, Maxwell & Faulkner, 1972). Also, van Den Hende, Muylle, Oyaert & de Roose (1972) report a steady increase in the proportion of SDHase low fibres in the longissimus of Pietrain and Landrace pigs, from 55% in pigs weighing 2 kg to 85% in pigs of 105 kg liveweight. The number of fibres in the SDHase high bundles in their published photograph appears even less than that published by Cooper et al. (1970) for the longissimus of the Poland China and Yorkshire breeds. The difference between the results of these two investigations on the porcine longissimus and those of Moody & Cassens (1968) and the present study, suggests a large source of variation in the histochemical properties of this muscle between pigs of different breeding and environment.

The present study shows that in Large White pigs between 10 and 56 days of age, a fast-twitch, purely aerobic fibre type is present in the diaphragm in greater proportions than in more mature pigs. This fibre type is predominant in the mouse diaphragm, occurs as 25% of fibres in the rat diaphragm, but does not occur in significant proportions in the diaphragms of larger animals (Davies & Gunn, 1972). These intraspecies and interspecies observations suggest that the diaphragm of larger animals increases its capacity for anaerobic metabolism.

However, the problem of metabolic adaption of muscle to increased body size awaits a comprehensive biochemical study of postnatal growth over a wide weight range.

2.4.8 Mechanism of fibre type changes

After an initial differentiation of fibres with respect to the extent to which they derive energy for contraction by aerobic or anaerobic metabolism, the most significant postnatal histochemical changes seen in this study are an increase in the proportion of myosin ATPase low fibres, and a concomitant increase in the proportion of GPase low fibres in longissimus (Table 5). A relative increase in a particular fibre type could occur either by:-

(i) the addition of new fibres to the population;

(ii) the subtraction of fibres of a different type from the population;

(iii) a rearrangement of fibre architecture to enable more fibres of one type to be seen in a transverse section than previously; or

(iv) a conversion of a fibre of one type into another.

Unless (i) and (ii) occur simultaneously, or the rearrangement in (iii) is particularly complex, only (iv) can occur without alteration to the number of fibres in a muscle. Estimates of the fibre populations of muscles during growth suggest that although fibre numbers may increase for a short neonatal period, subsequent postnatal growth of muscle is due to hypertrophy of fibres present at birth. This is evident in m. radialis of the rat (Morpurgo, 1898), for the human sartorius (MacCallum, 1898; Montgomery, 1962), the biceps brachii of the mouse (Goldspink, 1962), the biceps brachii, extensor carpi radialis, gastrocnemius and tibialis anterior of the rat (Enesco & Puddy, 1964), and the extensor carpi radialis, soleus and plantaris of the rat (Chiakulas & Pauly, 1965).

A significant decrease in the total number of fibres during growth has been reported in the plantaris muscle of the guinea-pig between 6 and 45 weeks of age (Faulkner et al., 1971; Faulkner, Maxwell & Lieberman, 1972) and in the longissimus and semitendinosus of the ox between 12 and 24 months of age (Bendall & Voyle, 1967). However, since neither of these investigations has accounted for the increasing covariance of mean fibre TSA and the TSA of a whole muscle that is inevitable when a series of animals of increasing body size are compared, these observations are questionable, and in any case do not examine the fibre population of animals at a time when the greatest increase in body size is occurring. The findings of Staun (1963) and the present results suggest that the postnatal fibre population of the longissimus of the pig is constant. Karpati & Engel (1967b) show that while the proportion of myosin ATPase low fibres in the guinea-pig soleus increases from 60 to 100% of the fibre population between birth and adulthood, the total fibre population increases only from 3,384 to 3,531.

Although the addition, subtraction or rearrangement of fibres is not disproved, it appears much more likely that a conversion of myosin ATPase high to myosin ATPase low fibres occurs with growth. The following aspects of the present study support this. In both longissimus and diaphragm, the TSA distribution and morphology of myosin ATPase low fibres at any stage of growth does not suggest a development from small, immature fibres (Fig. 17). The splitting of aerobic fibres and degeneration of anaerobic fibres seen in the longissimus of growing pigs by Todorov & Petrov (1969) has not been seen in the material used in present study. However, in pigs from birth to 15 kg liveweight, coincident with the most rapid increase in the proportion of myosin

ATPase low fibres (Fig. 32), many fibres of intermediate staining for the myosin ATPase reaction are seen in both muscles (Figs. 26, 33, 36). When the pH of the incubation medium is held at 9·5, intermediate reactions are seldom seen in more mature porcine muscle (Figs. 5, 8). The histochemical profile and mean TSA of these fibres suggest that they are in transition from fast-twitch combined aerobic and anaerobic fibres to slow-twitch purely aerobic fibres. They are located adjacent to myosin ATPase low bundles; the process would therefore augment the number of fibres in these bundles.

The possibility that a change in the characteristics of a muscle fibre as basic as the ATPase activity of its myosin and its intrinsic speed of contraction occurs with growth is of great interest, since it implies a considerable readjustment of the pattern of innervation and the architecture of the motor unit.

3.0 Part 2: POSTNATAL CHANGES IN MUSCLE DISTRIBUTION

3.1 INTRODUCTION

3.1.1 The relation between muscle function and muscle distribution

Part 1 of this thesis proposes a mechanism by which an animal adapts to support an increasing body mass; postural muscles oppose an increasing gravitational force by a proportionate increase in fibres best suited for the maintenance of posture. Part 2 proposes an adaptive mechanism for propulsive acceleration in the larger animal. For this acceleration to remain constant during growth, the muscular force developed must be proportional to the body mass; this can only be achieved if the TSA of propulsive isotonically contracting muscles increases in proportion to the weight of the animal, or in other words if the weight of propulsive muscles increases at a rate proportional to the 3/2 power of body weight. Disproportionate growth of this magnitude would be difficult to achieve, and so a constant acceleration is not attained. Nevertheless, preferential development of muscles best anatomically located for propulsive effort would be advantageous to the larger animal.

The pig is a suitable subject for an investigation of this problem. The extent of its postnatal growth exceeds that of most other domestic or laboratory animals (Table 8). Since the skeletal muscles of the pig support and propel its body almost from birth, they must adapt to accelerate a mass which increases a hundred-fold. Part 2 tests the hypothesis by determining patterns of growth among individual muscle units of the porcine carcass. The functional significance of the differences in growth of these muscles remains largely a matter for conjecture, although the histochemical methods used in Part 1 could provide some clarification.

Table 8. Relation of maternal weight to neonatal weight for several species of eutherian mammals.

Species and breed	Maternal weight (kg)	Neonatal weight (kg)	Maternal weight / Neonatal weight	Reference
Horse (Shetland)	191	19·6	9·2	Walton & Hammond (1938)
(Shire)	797	71·0	11·2	Walton & Hammond (1938)
Guinea-pig	1·02	0·094	10·9	Altman & Dittmer (1962)
Ox (Hereford)	514	32·1	16·0	Vaccaro & Dillard (1966)
(Holstein)	679	40·0	17·0	Altman & Dittmer (1962)
Man (U.S., European)	56	3·3	17·0	Altman & Dittmer (1962)
Monkey (Rhesus)	8·0	0·47	17·0	Altman & Dittmer (1962)
Goat (Saanen)	70·1	3·14	22·3	Altman & Dittmer (1962)
Sheep (Suffolk, twin lamb)	94·4	4·22	22·4	Hammond (1932)
Cat	2·45	0·104	23·5	Altman & Dittmer (1962)
Dog (Beagle)	8·5	0·30	28·3	Hwai-Ping & Huggins (1971)
Mouse (Piebald)	0·0364	0·00126	28·9	Altman & Dittmer (1962)
Indian elephant	3000	90	33	Burton (1965)
Blue whale	150000	4100	36·6	Burton (1965)
Common shrew	0·0060	0·00015	40	Barrett (1960)
Rat (Wistar)	0·243	0·00530	45·8	Altman & Dittmer (1962)
Rabbit (New Zealand White)	4·08	0·065	62·8	Altman & Dittmer (1962)
Pig (German Landrace)	141	1·40	101	Haring et al. (1966)
(Vietnamese Miniature)	44·0	0·34	129	Haring et al. (1966)
Polar bear	160	0·23	690	Burton (1965)

3.1.2 Individual muscles as growth units

The musculature of the body is divided into individual muscles in order to direct contractile activity between specific skeletal points. The development of this localised contractile activity at any particular growth stage is conveniently estimated by the weight of a dissected muscle. The use of individual muscles to test a hypothesis relating muscle distribution and function, however, depends on the extent to which functional units within a muscle have similar origins and insertions, and contract at similar rates. M. semitendinosus, for instance, has a deep region with a relatively high

proportion of myosin ATPase low fibres (Davies & Gunn, 1971); if the hypothesis is well founded, the relative growth of the entire semitendinosus would not therefore be typical of either a purely postural or a purely propulsive muscle. Similarly, the extent to which adjacent muscles act over different joints and contract at different rates will determine whether muscles can be grouped in order to simplify the data obtained from dissection studies. The dissection of individual muscles, as used in this study, is nevertheless the only practical way of dividing the entire muscular system of an animal into functional groups.

Walker (1961) and Dumont, Le Guelte & Arnoux (1961a, b) were the first to use a total muscle dissection method on cattle. This work was continued by Butterfield (1962, 1964a) and Berg & Mukhoty (1970). The method has also been used on sheep (Lohse, Moss & Butterfield, 1971) and on pigs (Cuthbertson & Pomeroy, 1962; Dumont, Schmitt & Roy, 1969; Richmond & Berg, 1971b). The growth of individual muscles has been described for cattle (Butterfield & Berg, 1966a)and for sheep (Lohse, Moss & Butterfield, 1971), but not previously for pigs.

3.1.3 Methods for studying relative growth

The growth of muscle as a function of time is important equally to the pigman whose costs of production are closely related to time, and the biologist interested in the rate of synthesis of muscle protein. However, growth as a function of time is, in the same way as any chemical reaction, strongly influenced by factors external to the system under consideration. The investigator comparing the rates of growth of two body components y_1 and y_2 under different conditions must seek a reference parameter equally affected by external factors irrelevant to the conditions under consideration.

Thus if y_1 and y_2 are the weights of the same muscle in two breeds of pig, the breed difference between y_1 and y_2 is ideally estimated by comparing the relationship between y_1 and x_1 and between y_2 and x_2, where x_1 and x_2 are the weights of another body component such that only a genetic difference will change these relationships. In order to meet this criterion for the choice of an independent variate, it is usual for x to be the weight of a tissue (for example, total muscle) of which y is a part (for example, the weight of an individual muscle). This choice confers no guarantee that influences other than those of genetic origin will not affect the relationships studied.

The relationship derived between y and x is an important consideration. The method used in the present study presupposes that growth is an exponential rather than a linear phenomenon, an assumption based on the following two concepts.

(1) During exponential growth, at any instant the rate of change in the dimension of a body component x is proportional to its value at that instant,

$$\text{i.e.} \quad \frac{dx}{dt} = k.x$$

or by integration $\ln x = \ln a + k.t$

where a is the value of x when time $t = 0$, and k is a constant. Thus when the natural logarithm of x is plotted against time t, the presence of a straight line indicates exponential growth. Brody (1945) defines k as the instantaneous relative growth rate, and shows that k is constant over intervals of the pre- and postnatal growth of the rat, as it is for bacterial and even human populations under certain conditions. At some stages, the growth of an organism or population is analogous to a chemical reaction complying with the

law of mass action. In a finite universe, all such reactions are of limited duration. Brody (1927) and Goedbloed (1972) analyse data suggesting that abrupt changes occur in the value of k during growth of rats and mice, resulting in a step-like decline in growth rate towards maturity. Laird, Tyler & Barton (1965) suggest that during pre- and postnatal growth in body weight of the guinea-pig, k decreases exponentially with age. The value of k, whether showing sudden changes or declining steadily, is nevertheless meaningful biologically since it represents the rate of multiplicative growth of living substance.

(2) The ratio b of the instantaneous relative growth rates k_1 and k_2 of any two body components x and y is constant over limited ranges (Huxley, 1932; Brody, 1945). Thus

$$b = \frac{k_2}{k_1} = \frac{\frac{dy}{dt} \cdot \frac{1}{y}}{\frac{dx}{dt} \cdot \frac{1}{x}} = \frac{\frac{dy}{y}}{\frac{dx}{x}}$$

and by integration,

$$\ln y = \ln a + b. \ln x$$

where a is the value of y when $\ln x = 0$. When the natural logarithm of x is plotted against the natural logarithm of y, the presence of a straight line of gradient b demonstrates an "allometric" relationship between x and y;

$$y = a. x^b,$$

where b is the "differential growth ratio" (Huxley, 1924). When two body components grow in such a way that their rates of multiplicative growth, which need not be constant themselves, bear a constant relationship to one another, their relative growth is said to be allometric.

Miller & Weil (1963), Tulloh (1964) and Seebeck & Tulloh (1966) provide examples showing that an analysis of relative growth should take the multiplicative nature of growth into account. This is because arithmetic regressions on measurements of growing body components are not linear over any stage of growth, although the data may suggest it. The regression line does not pass through the origin, and extrapolations to younger animals are impossible. The covariance of x and y increases with x; calculation of the regression line, and a comparison of the values of b are not possible by simple statistical procedures. Also, it is essential that x and y have the same dimensions. But since the double logarithmic regression of x on y is frequently linear over wide weight ranges, the allometric equation has definite advantages in the study of relative growth. When a parabola or hyperbola, derived from a linear logarithmic regression, is fitted to the points plotted on arithmetic coordinates, it passes through the origin; extrapolations can therefore be made in some circumstances to young animals. The use of a double logarithmic regression is only valid when the variance of x enters the equation $y = a.x^b$ multiplicatively, rather than additively (Glass, 1969). Since growth is a multiplicative process, its variance is also multiplicative. By logarithmic transformation, therefore, the covariance of x and y becomes independent of x, enabling a simple statistical comparison of two growth ratios. It is also then possible to relate the growth of x and y when they are of different dimensions.

From the foregoing, it appears essential that the growth of two body components, relative to the growth of another component, should be compared by allometry. This applies both to a study of two components of the same animal,

and to a study of the same component of two animals under different genetic or environmental influences. This study has therefore been designed to provide data suitable for this type of analysis. The results have been analysed by the methods of covariance analysis originally used for a similar problem by Reeve (1940), enabling a comparison of the growth ratio b. The double logarithmic regression equations also provide a means of comparing the values of y for two groups of animals at a nominal value of x. The allometric equation is used for this purpose by Elsley, McDonald & Fowler (1964), Mukhoty & Berg (1971), Fowler & Livingstone (1972), and in the present study.

3.1.4 The relation between muscle development and meat quality

Postmortem glycolytic rates, and the consequent development of a condition characterised by pale, soft and exudative (PSE) muscles, vary between breeds and strains of pigs (Judge, Cahill, Kunkle & Bruner, 1959; Lawrie & Gatherum, 1962; Allen, Forrest, Chapman, First, Brag & Briskey, 1966). Since this phenomenon is important to the production and processing of pig meat, much effort has been expended to determine its etiology. It has been the subject of two extensive reviews (Briskey, 1964; Bendall & Lawrie, 1964) and three international symposia (Sybesma, van der Wal & Walstra, 1969; Hessel-deHeer, Schmidt, Sybesma & van der Wal, 1971; Cassens, Giesler & Kolb, 1972). It is well established that the environmental temperature before and after slaughter, and the amount of pre-slaughter exercise, influences the rate of postmortem glycolysis (Briskey, Forrest & Judge, 1966; Forrest, Will, Schmidt, Judge & Briskey, 1968; Kallweit, 1969; Lendfers, 1969). Also important is the level of anoxia (Lister, Sair, Will, Schmidt, Cassens, Hoekstra & Briskey, 1970) and nervous stimulation (Bendall, 1966; McLoughlin & Tarrant, 1969; Lister & Ratcliff,

1971; Sair, Kastenschmidt, Cassens & Briskey, 1972) of the muscle at slaughter. Adrenal and thyroid activities appear to be related to the development of the syndrome (Ludvigsen, 1957a; 1960; Topel, Merkel & Wismer-Pedersen, 1967; Judge, Briskey, Cassens, Forrest & Meyer, 1968; Topel, 1969; Judge & Marpel, 1971; Lister, 1971). These findings do not, however, indicate a fundamental reason for the differences between strains of pigs, or why the incidence of the condition appears to be increasing (Ludvigsen, 1957b; Lister, 1971; Eikelenboom, 1972).

The observations of Vold, Steinhauf & Weniger (1965), Jensen, Craig & Robison (1967), Topel, Merkel & Wismer-Pedersen (1967), Flock (1968), Judge, Forrest, Sink & Briskey (1968), Wismer-Pedersen (1968), Steinhauf (1969), Dildey, Aberle, Forrest & Judge (1970), Charpentier, Monin & Ollivier (1971) and Unshelm, Kallweit, Oldigs, Schröder, Pfeiderer & Schutzbar (1972) suggest that the incidence of high postmortem glycolytic rates is a consequence of the development of muscular breeds of pigs to satisfy the increasing consumer demand for high protein, low fat products. It is expected that the limitations of the circulatory system to supply nutrients and oxygen to a muscle mass not essential to an animal's normal functional requirements will necessitate that less frequently used muscles will obtain energy for contraction from an intrinsic store of glycogen, and that they will metabolise this anaerobically. Since muscles having a predominantly anaerobic metabolism develop a more severe PSE condition than those with a predominantly aerobic metabolism (Beecher, Cassens, Hoekstra & Briskey, 1965; Beecher, Kastenschmidt, Cassens, Hoekstra & Briskey, 1968), it is expected that a high proportion of relatively anaerobic muscles will enhance any tendency to rapid postmortem glycolysis.

Any work to determine the extent to which modern pig breeding is contributing to poor meat quality will require an understanding of the morphological differences between highly muscular and less muscular strains of pigs, and the manner in which these differences arise. There has been no previous report of a study comparing between two breeds of pigs the relative growth of individual muscles and muscle groups, and the weights of muscle groups at identical body weights. It is therefore appropriate to compare in this way the growth of muscles and other major tissues of the British Large White pig with a breed in which muscularity and poor meat quality are well recognised characteristics. This is the main object of Part 2 of this thesis. The use of the Belgian Pietrain pig for this comparison is justified in a discussion in paragraph 3.4.2, page 110. Data on bone, fat and visceral organ growth have also been analysed. Should this study demonstrate a significant difference between breeds in the relative development of individual muscles, an attempt to test the above hypothesis using the histochemical methods of Part 1 would also be appropriate. However, the Pietrains were dissected at a stage of the work when the techniques for the histochemical demonstration of fibre types and their profiles were only partly developed and their relevance not fully appreciated. Some histochemistry was nevertheless attempted, and the results are included in this part of the thesis.

3.2 MATERIALS AND METHODS

3.2.1 Source and initial preparation of material

Thirty-six female pigs of the Large White and Pietrain breeds, with liveweights ranging from 1·3 to 73 kg (2 to 214 days of age) were obtained from the School of Agriculture, University of Newcastle-upon-Tyne. The Pietrains were obtained during August and September 1970, from stock originally imported to Britain from Belgium in 1964. The Large White pigs, obtained during August and September 1971, were from a herd in which intense selection for lean meat production had been practised between 1963 and 1969, based on growth rates, ultrasonic fat depths and feed conversion ratios. The Large White pigs provided samples for histochemistry and dissection data used in Part 1 of this thesis. Each breed was chosen to include as nearly as possible three pigs of each of the following liveweights: 2, 4, 8, 16, 32 and 64 kg. Pigs of 32 and 64 kg liveweight were killed near Newcastle and brought to Edinburgh as dressed carcasses. Smaller pigs were brought alive to Edinburgh where they were killed and eviscerated by a simulated abattoir procedure. The heart, spleen, liver, uterine horns and ovaries, stomach, small intestine, large intestine and gut content were weighed. Subtraction of the weight of gut content from liveweight gave an estimation of empty body weight (EBW). The carcasses were weighed and stored at a temperature between $0^{o}C$ and $4^{o}C$ for up to 5 days before dissection.

Since animals of each breed were obtained in different years, and the author had no control over their environment, this study compares the growth of body components relative to each other, rather than their time rate of growth.

3.2.2 Dissection procedure

3.2.2.1 Division of the carcass

With the head still attached, soft tissues in the median sagittal plane were cut with a knife. The head and vertebral column were then bisected with a handsaw. The right half of the carcass was used for dissection in each case. Abdominal fat, kidney, adrenal gland, diaphragm, m. rectus abdominis and m. transversus abdominis were removed and weighed. Weights to three significant figures were recorded on a cyclostyled worksheet. Appendix 1 is an example of the records made during the dissection of one half carcass. The anatomical nomenclature used follows the recommendations of Nomina Anatomica Veterinaria (1968). The carcass was divided into 'forequarter' and 'hindquarter' by cutting it transversely at the thoracolumbar junction, after reflection of the cranial end of m. psoas major and m. psoas minor, and removal of the diaphragm, m. transversus abdominis and m. rectus abdominis. Only m. longissimus, m. obliquus abdominis and m. iliocostalis were therefore transected. The diaphragms of the commercially slaughtered pigs were frequently cut during evisceration, thus precluding a satisfactory growth study of this muscle. Both quarters were weighed, and the forequarter was returned to the coldroom.

3.2.2.2 Dissection of the hindquarter

M. obliquus internus abdominis and the caudal portion of m. obliquus externus abdominis were dissected out. Skin, subcutaneous fat and m. cutaneus were removed. M. cutaneus was dissected away, and the fat was stripped from the skin with a butcher's knife. The skin was not removed from the head or the digits. Muscles were separated from the carcass in the

order shown in Appendix 1. They were placed on a damp paper towel under plastic sheeting, identified by a number corresponding to that on the worksheet. As soon as possible, muscles were cleaned of intermuscular fat, fascia and tendon, and were weighed. Intermuscular fat and fascia were collected in a polythene bag for weighing on completion of the hindquarter dissection, as was tendon and scrap (blood vessels, nerves and lymph nodes). Skin, digits and tail were weighed as one entity. It was considered impractical to dissect the deep spinal muscles as separate entities and these were therefore grouped under one heading. Whenever it was discovered that errors were introduced in separating certain muscles, these were grouped for weighing.

Bones were cleaned and weighed. Measurements were made, width being the minimum craniocaudal dimension and length as follows:-

Femur: Apex of trochanter major to distal articular surface of lateral condyle.

Tibia: Tuberosity to medial malleolus.

Metatarsus IV: Proximal to distal extremity.

3.2.2.3 Dissection of forequarter

Skin, subcutaneous fat and m. cutaneus were removed as for the hindquarter. Following dissection of the neck muscles, the half head was removed and weighed. Portions of the salivary glands remaining on the neck were included as scrap. Deep cervical and spinal muscles were incorporated as one group, and thoracic muscles in another. Bone lengths were measured as follows:-

Scapula: Caudal angle to tuber supraglenoidale.

Humerus: Apex of tuberculum majus pars cranialis to distal articular surface of lateral condyle.

Radius and Ulna: Tuber olecrani to distal extremity of radius.

Metacarpus IV: Proximal to distal extremity.

Ribs I and VIII: Direct distance between the head and costochondral junction.

The muscles of mastication were exposed by removal of the supraorbital process of the frontal bone, and the zygomatic arch. They were dissected from the skull. The seven muscles removed from the head, and those remaining undissected, were not included in the estimation of total side muscle (TSM).

3.2.3 Analysis of data

The data for each organ, muscle and bone were arranged in order of increasing body weight. All data obtained are given in this form in Appendix 2. The parameters of the double logarithmic regression between two carcass components x and y were estimated by the methods for linear regressions outlined by Diem & Lentner (1970). The regression coefficient b is the differential growth ratio of the component y relative to x; where this is significantly greater, not significantly different, or significantly less than 1, the 'impetus' to growth of y is described as 'high', 'average' or 'low' respectively. The calculations of the values and standard deviations of b, and the significance of the difference between b and 1, were assisted by on-line computer facilities. Where appropriate, the significance of the

difference between values of b for two body components was tested at the 5% level by the test quotient t.

The allometric equations enabled the calculation of the value of log y and its variance for a given value of log x. The significance of the difference between two values of log y was compared either by:

(1) comparing the 95% or the 99% confidence limits for the values of log y; when these limits do not overlap, the values of log y and therefore y are significantly different, or

(2) calculating the 95% or the 99% confidence limits of the difference between the two values of log y; when these limits are above or below zero, the ratio of the values of y is significantly different from 1, i.e. the values of y are significantly different.

3.2.4 Histology of m. longissimus

The methods used for the histological processing and microscopic measurements of m. longissimus of the Large White pigs has been described in Part 1. Samples of the left longissimus muscle in the dorsomedial region at the thoracolumbar junction (as for the Large White pigs) were removed from each of the 18 Pietrain pigs, frozen, sectioned and incubated to demonstrate the activity of myosin ATPase. Two of these samples were subsequently considered unsuitable for study. Additional histological material, sampled at an abattoir near Newcastle, was obtained from 4 pigs of mean liveweight 97·2 kg, from the same herd as the other Pietrains. Since this supply was subsequently discontinued, samples equivalent in number to that obtained from Large White pigs (the 16 pigs of Series 2) were not examined. Thus samples from 20 Pietrain and 34 Large White pigs were used for histological study.

Mean fibre TSA was estimated by counting the fibres in a known area of the section. At the same time, an estimate of the proportion of myosin ATPase low fibres was made. The width and depth (but not TSA) of the muscle at the thoracolumbar junction were measured; these values were used to estimate TSA.

3.3 RESULTS

3.3.1 Growth of viscera and carcass

Table 9 lists allometric equations comparing the growth of viscera and carcass with that of empty body weight (EBW). There are significant differences between Pietrains and Large Whites only in the relative growth of the intestines and the reproductive system. Intestinal growth is lower, and the growth of uterus and ovaries is higher in the Pietrain. All the visceral organs studied, except the uterus and spleen, and the lung which was weighed for the Large White only, develop at a rate significantly less than EBW in one or other of the two breeds. In Table 10a, the allometric

Table 9. Regression equations comparing the growth of viscera and carcass relative to empty body weight between 18 Pietrain and 18 Large White female pigs from birth to 64 kg liveweight.

Weight of y = a.(empty body weight)^b

Component y	PIETRAIN Growth ratio b*	s_b	log a	Impetus	LARGE WHITE Growth ratio b*	s_b	log a	Impetus	Sig. of diff.**
Heart	0·824	0·022	-1·586	low	0·847	0·024	-1·618	low	NS
Lung	not measured				0·985	0·042	-1·760	average	-
Liver	0·877	0·046	-1·124	low	0·856	0·092	-0·955	average	NS
Spleen	0·955	0·048	-2·571	average	0·914	0·063	-2·312	average	NS
Intestines	0·822	0·035	-0·776	low	1·059	0·069	-1·403	average	$P < 0·025$
Uterus and ovaries	1·331	0·097	-4·464	high	0·950	0·149	-3·011	average	$P < 0·05$
Right kidney	0·839	0·020	-1·966	low	0·844	0·029	-1·959	low	NS
Right adrenal gland	0·676	0·076	-3·115	low	0·730	0·037	-3·329	low	NS
Half carcass	0·984	0·011	-0·320	average	1·003	0·014	-0·401	average	NS

* Regression coefficient b, standard deviation s_b.
** Significance of the difference in b between breeds, tested at the 5% level by the test quotient t; NS = not significant.

Table 10. Analysis of Pietrain and Large White pigs at empty body weights (EBW) of 2 and 60 kg.

Component y	EBW 2 kg Pietrain	EBW 2 kg Large White	EBW 2 kg Ratio*	EBW 2 kg Sig.**	EBW 60 kg Pietrain	EBW 60 kg Large White	EBW 60 kg Ratio*	EBW 60 kg Sig.**
(a) Weight of viscera and half carcass y (in grammes) calculated from regressions of the form $y = a.(EBW)^b$, using values of a and b from Table 9.								
Heart	13·6	15·1	0·90	NS	224	286	0·84	NS
Liver	59·0	74·3	0·79	NS	1160	1360	0·85	NS
Spleen	3·81	5·07	0·75	NS	98·2	113	0·87	NS
Intestines	136	124	1·10	NS	2740	4540	0·60	NS
Uterus and ovaries	0·851	1·33	0·64	NS	78·7	33·7	2·34	NS
Right kidney	6·37	6·71	0·95	NS	110	119	0·92	NS
Right adrenal gland	0·131	0·120	1·09	NS	1·30	1·44	0·90	NS
Half carcass (including ½ head)	847	811	1·04	NS	24100	24600	0·98	NS
(b) Weight of tissues y (in grammes) calculated from regressions of the form $y = a.(\text{half carcass})^b$, using weights of half carcass from (a) above, and values of a and b from Table 11.								
Total side muscle	378	313	1·21	P < 0·001	12900	11900	1·08	P < 0·05
Total side bone	136	134	1·01	NS	2240	3130	0·72	P < 0·001
Total side fat	73·8	99·3	0·74	NS	4290	4620	0·93	NS

*Ratio of component weights: $\frac{\text{weight of y for Pietrain}}{\text{weight of y for Large White}}$

In Table 10c, ratios not followed by the same letter are significantly different ($P < 0·05$).

**Significance of the difference of this ratio from 1, tested at the 5% level by the test quotient t; NS = not significant.

Table 10 (continued)

Component y	EBW 2 kg Pietrain	EBW 2 kg Large White	EBW 2 kg Ratio*	EBW 2 kg Sig.**	EBW 60 kg Pietrain	EBW 60 kg Large White	EBW 60 kg Ratio*	EBW 60 kg Sig.**
(c) Weight of heart and muscle groups y (in grammes) calculated from regressions of the form $y = a.(TSM)^b$, using weights of TSM from (b) above, and values of a and b from Table 14.								
Abdomen	22·9	14·2	1·61	P < 0·001	918	673	1·34 A	P < 0·001
Thigh	111	90·4	1·23	P < 0·001	4530	3840	1·18 BC	P < 0·001
M. longissimus	27·8	24·1	1·15	P < 0·001	1450	1200	1·16 BC	P < 0·001
Neck	19·9	14·1	1·41	P < 0·001	509	459	1·11 ABCD	NS
Crus	18·2	15·6	1·17	P < 0·001	614	567	1·08 BCD	NS
Pectoral girdle	58·3	50·7	1·15	P < 0·001	1690	1580	1·07 BCD	NS
Brachium	56·0	42·8	1·31	P < 0·001	1400	1370	1·02 D	NS
Rostrum	2·19	1·65	1·33	P < 0·001	31·3	31·3	1·00 DE	NS
Mandible	7·74	6·76	1·14	P < 0·05	178	181	0·98 DE	NS
Antebrachium	11·3	9·86	1·15	P < 0·001	238	242	0·98 DE	NS
Heart	13·7	15·1	0·91	NS	226	267	0·85 E	P < 0·001
(d) Weight of bones y (in grammes) calculated from regressions of the form $y = a.(TSB)^b$, using weights of TSB from (b) above, and values of a and b from Table 16.								
Cranial axial	50·0	48·7	1·03	NS	813	1120	0·73	P < 0·001
Scapula	5·78	5·77	1·00	NS	119	167	0·71	P < 0·001
Humerus	10·6	11·3	0·94	NS	187	235	0·80	P < 0·001
Radiua & ulna	8·85	9·06	0·98	NS	134	196	0·68	P < 0·001
Carpus & metacarpus	6·24	7·33	0·85	P < 0·001	84·3	129	0·65	P < 0·001
Caudal axial	17·8	19·1	0·93	NS	372	525	0·71	P < 0·001
Femur	11·5	11·4	1·01	NS	218	296	0·74	P < 0·001
Patella	0·643	0·604	1·06	NS	13·5	19·3	0·70	P < 0·001
Tibia & fibula	9·59	9·48	1·01	NS	152	212	0·72	P < 0·001
Tarsus & metatarsus	12·0	11·7	1·03	NS	151	220	0·69	P < 0·001
Total bones	133	135			2240	3120		

equations have been used to compare between breeds the weights of organs and carcass of pigs with EBW's of 2 and 60 kg. There are no significant differences in these weights between breeds.

3.3.2 Growth of major tissues (muscle, bone and fat)

Allometric equations describing the growth of total side muscle (TSM), total side bone (TSB) and total side fat (TSF), relative to the growth of the half carcass, are shown in Table 11 and Fig. 39. TSM and TSB maintain a constant allometric relationship with the carcass over the period of growth studied. Fat growth is more variable. Growth over the period studied is greatest for fat, although the growth of this tissue is significantly greater

Table 11. Regression equations comparing the growth of total side fat, total side muscle and total side bone, relative to half carcass weight, between 18 Pietrain and 18 Large White female pigs from birth to 64 kg liveweight.

Weight of y = a.(half carcass weight)^b

Tissue y***	PIETRAIN Growth ratio b*	s_b	log a	Impetus	LARGE WHITE Growth ratio b*	s_b	log a	Impetus	Sig. of Diff.**
Total side fat	1·214 A	0·048	-1·686	high	1·126 A	0·078	-1·279	average	NS
Total side muscle	1·054 B	0·010	-0·509	high	1·067 A	0·009	-0·609	high	NS
Total side bone	0·842 C	0·018	-0·340	low	0·924 B	0·021	-0·561	low	P < 0·01

*Regression coefficient b, standard deviation s_b.
Values of b within each breed not followed by the same letter are significantly (P < 0·05) different between tissues.
**Significance of the difference in b between breeds, tested at the 5% level by the test quotient t; NS = not significant.
***Not including tissues of head and tail.

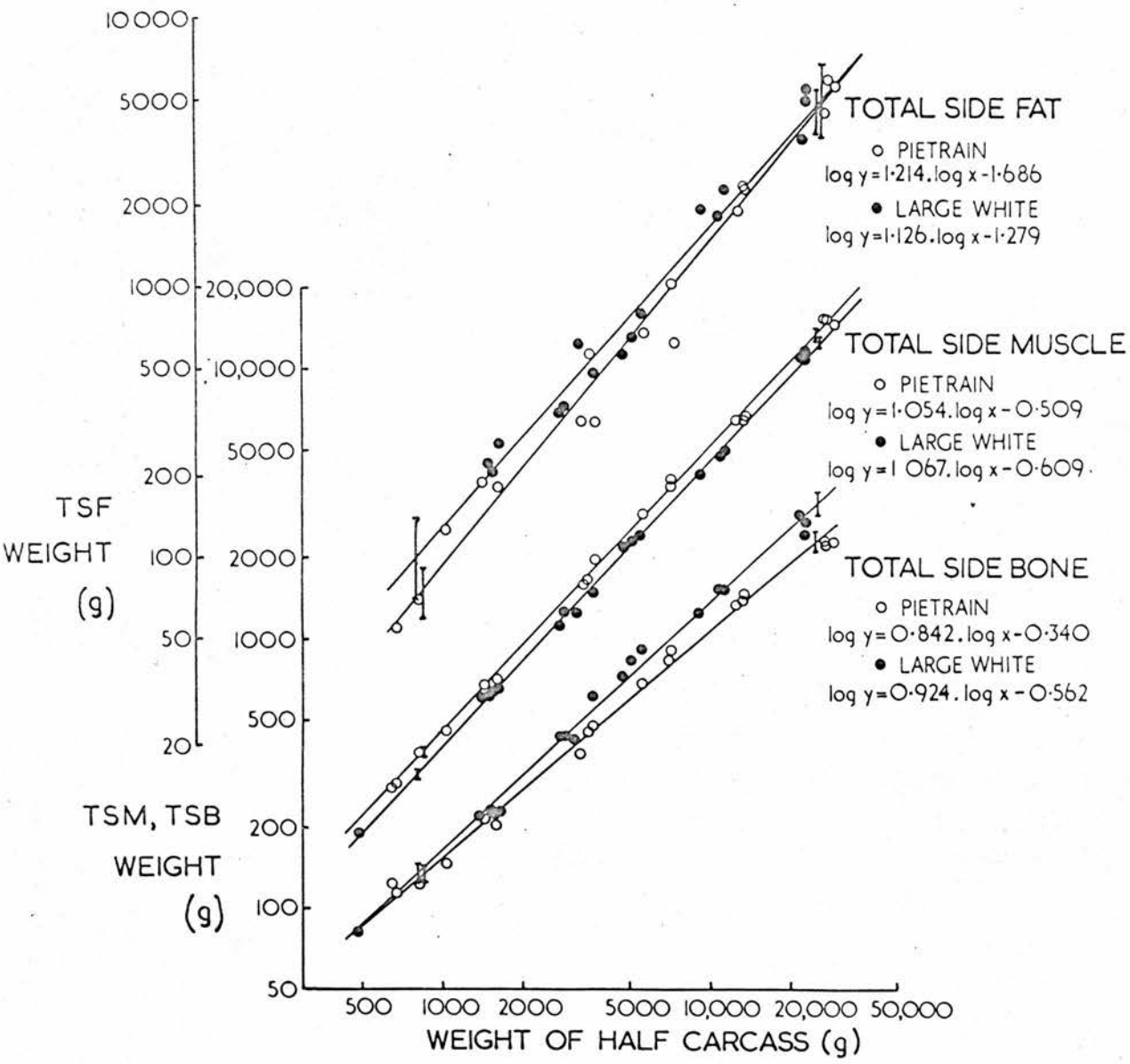


Fig. 39. Allometric equations comparing the growth of total side fat (TSF), total side muscle (TSM) and total side bone (TSB) relative to half carcass weight, between Pietrain and Large White female pigs.

I 95% confidence limits for tissue weights in pigs of 2 and 60 kg empty body weight.

than overall carcass growth only for the Pietrain. Muscle growth is significantly higher, and bone growth significantly lower, than overall carcass growth for both breeds. The growth of bone relative to the carcass is significantly greater for the Large White than for the Pietrain. The growth of muscle and fat is not significantly different between the two breeds.

The allometric equations in Table 11 enable the comparison of body composition between the two breeds at the same EBW, by using the values for half carcass weight shown in Table 10a. The weights of the three major tissues in pigs weighing 2 and 60 kg are compared in Table 10b and Fig. 39. The Pietrain of 2 kg EBW has significantly more muscle than the Large White pig at the same EBW. Bone and fat weights are not significantly different. At 60 kg EBW, muscle is still significantly better developed, but the weight of bone is now significantly lower in the Pietrain. The higher weight of fat in the Large White of 60 kg EBW is not significant.

Although the growth of fat relative to the carcass is similar for the two breeds, it is possible that the variable growth of this tissue obscures the relationship between muscle and bone in the carcass. Therefore the double logarithmic regressions of TSM and TSB against total side muscle plus bone (TSM+B) have been studied. In addition, data obtained by McMeekan (1940b, c) has been used to compare the "typical British commercial Large White pigs" used in his work with a modern 'improved' strain. The regressions are shown plotted in Fig. 40. The regression equations are given in Table 12. The growth of muscle relative to muscle plus bone is not significantly different between the Pietrains and Large Whites used in the

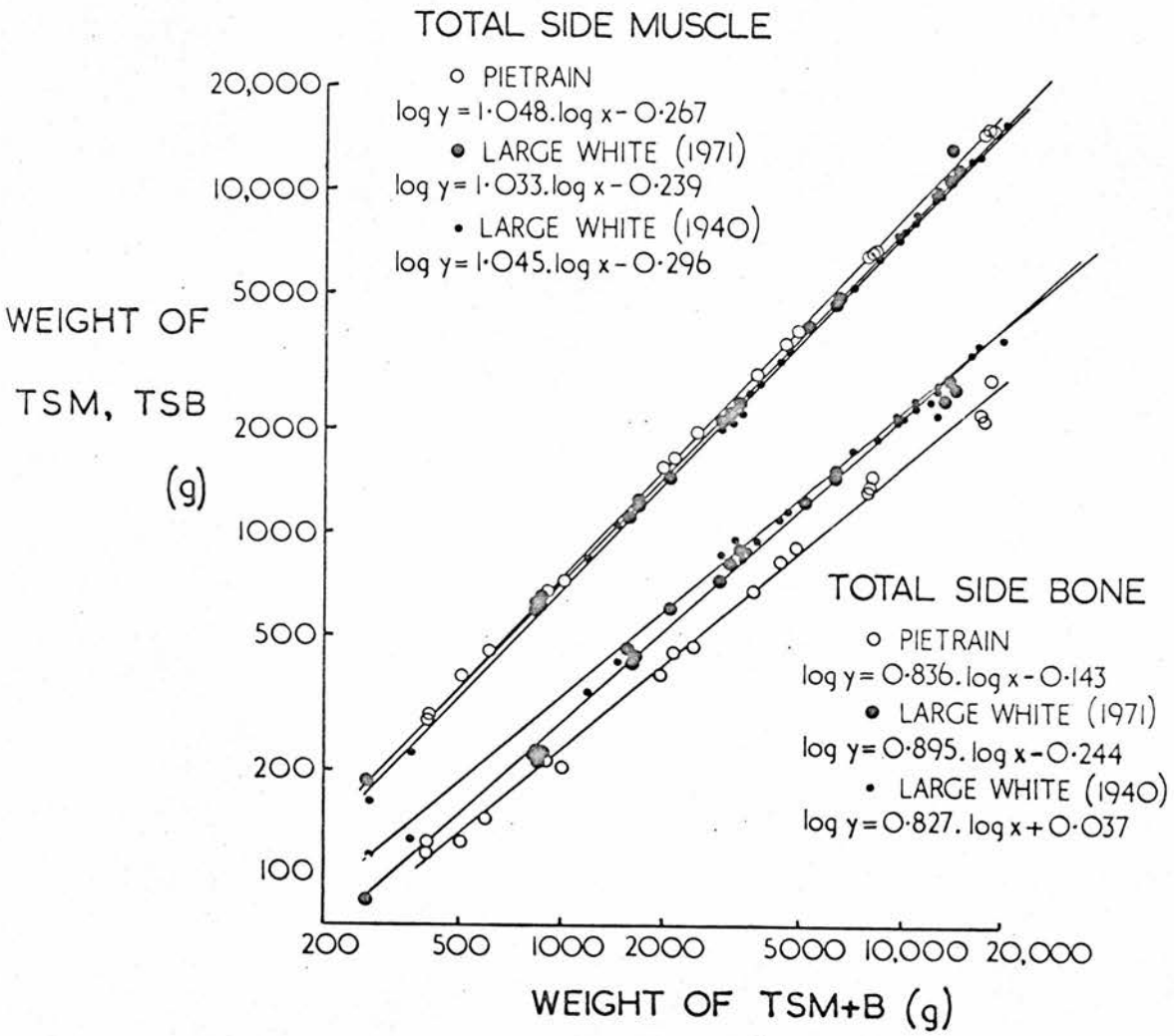


Fig. 40. Allometric equations comparing the growth of total side muscle (TSM) and total side bone (TSB), relative to total side muscle plus bone (TSM+B), between the Pietrain and Large White pigs of the present study and the Large White pigs dissected by McMeekan (1940b, c).

Table 12. Regression equations comparing the growth of total side muscle and total side bone relative to total side muscle plus bone between 18 Pietrain and 18 Large White female pigs from birth to 64 kg liveweight, and between 25 Large White pigs of mixed sexes from birth to 100 kg liveweight dissected by McMeekan (1940b, c).

Weight of $y = a.(\text{Total side muscle plus bone})^b$

	y = Total side muscle				y = Total side bone			
Breed	Growth ratio b*	s_b	log a	Impetus	Growth ratio b*	s_b	log a	Impetus
Pietrain	1·048 A	0·005	−0·267	high	0·836 A	0·015	−0·143	low
Large White (1971)	1·033 A	0·005	−0·239	high	0·895 B	0·018	−0·244	low
Large White (1940)	1·045 A	0·040	−0·296	average	0·827 AB	0·034	+0·037	low

*Regression coefficient b, standard deviation s_b.
Values of b within each tissue not followed by the same letter are significantly ($P < 0·05$) different between breeds.

Table 13. Muscle:bone ratios for Pietrain and Large White pigs at two values of total side muscle plus bone, calculated from the allometric equations given in Table 12.

	Total side muscle plus bone weight			
	1 kg		10 kg	
Breed	Muscle: bone ratio	95% limits*	Muscle: bone ratio	95% limits*
Pietrain	3·24	3·07 – 3·41	5·28	4·99 – 5·60
Large White (1971)	2·62	2·48 – 2·77	3·61	3·37 – 3·86
Large White (1940)	2·01	1·69 – 2·59	3·54	3·09 – 4·05

*Probability of a value outside these limits < 0·05.

present study, and between these and the Large Whites of 1940. The growth of bone relative to muscle plus bone is significant ($P < 0{\cdot}001$) only between Pietrains and modern Large Whites.

The ratio of TSM to TSB at any value of TSM+B, and its variance, is estimated from the difference between log TSM and log TSB. Muscle:bone ratios so calculated, and their 95% confidence limits, are shown in Table 13. Where the 95% confidence limits do not overlap, the differences between weights and breeds are significant. During an increase in TSM+B from 1 to 10 kg, there is a significant change in the muscle:bone ratios within the Pietrain and both groups of Large White pigs. The ratio is significantly higher for the Pietrain breed than for either Large White strain at both growth stages, but there is no significant difference between the Large Whites dissected by McMeekan (1940b, c) and those of the present study.

Differences between breeds will be smaller in the component making the largest contribution to TSM+B. It is not possible to conclude by comparing tissue growth with TSM+B growth whether either or both a greater development of muscle, or a lesser development of bone, contribute to the difference in muscle:bone ratio demonstrated.

3.3.3 Growth of muscle

Table 14 lists allometric equations comparing the growth of the heart, 18 muscle groups and 62 individual muscles with the growth of TSM. Muscles are grouped according to the articulations over which they act. The topographical distribution of individual muscles according to their growth ratios is indicated diagrammatically for each breed in Figs. 41 and 42.

Table 14. Regression equations comparing the growth of muscles and muscle groups relative to total side muscle in 18 Pietrain and 18 Large White female pigs from birth to 64 kg liveweight. Muscles are grouped according to the articulations over which they act.

Muscle weight = a.(Total side muscle)^b

Muscle	PIETRAIN Growth ratio b*	s_b	log a	Impetus	LARGE WHITE Growth ratio b*	s_b	log a	Impetus	Sig. of diff.**
HEART	0·794	0·021	-0·909	low	0·789	0·024	-0·790	low	NS
ROSTRUM	0·753	0·024	-1·600	low	0·808	0·023	-1·798	low	NS
MANDIBLE	0·890	0·018	-1·403	low	0·902	0·024	-1·420	low	NS
NECK	0·918	0·026	-1·067	low	0·956	0·028	-1·235	average	NS
PECTORAL GIRDLE									
Rhomboideus	0·819	0·038	-1·426	low	0·861	0·042	-1·551	low	NS
Trapezius	0·946	0·027	-1·738	average	0·948	0·048	-1·789	average	NS
Omotransversarius	0·952	0·090	-2·811	average	0·934	0·042	-2·713	average	NS
Brachiocephalicus	0·947	0·020	-1·774	low	0·930	0·018	-1·685	low	NS
Latissimus dorsi	0·927	0·018	-1·413	low	0·912	0·023	-1·341	low	NS
Serratus ventralis	1·015	0·022	-1·467	average	0·979	0·013	-1·366	average	NS
Mm. Pectorales	0·955	0·014	-1·176	low	0·974	0·016	-1·190	average	NS
Total pectoral girdle	0·953	0·009	-0·690	low	0·945	0·023	-0·653	low	NS
BRACHIUM									
Shoulder									
Supraspinatus	0·885	0·024	-1·244	low	0·952	0·018	-1·456	low	P < 0·05
Infraspinatus	0·968	0·021	-1·601	average	1·000	0·017	-1·731	average	NS
Subscapularis	0·909	0·031	-1·877	low	0·958	0·015	-1·981	low	NS
Teres major	0·982	0·027	-2·120	average	0·888	0·012	-1·835	low	P < 0·01
Teres minor	0·949	0·040	-2·494	average	0·996	0·045	-2·713	average	NS
Deltoideus	0·934	0·035	-2·172	average	0·927	0·021	-2·132	low	NS
Coracobrachialis	0·890	0·030	-2·603	low	0·933	0·026	-2·730	low	NS
Articularis humeri	0·971	0·040	-2·900	average	0·960	0·057	-2·973	average	NS
Total shoulder	0·928	0·012	-0·946	low	0·984	0·019	-1·144	average	P < 0·05

*Regression coefficient b, standard deviation s_b.
**Significance of the difference in b between breeds, tested at the 5% level by the test quotient t; NS = not significant.

Table 14 (continued)

Muscle	PIETRAIN Growth ratio b*	PIETRAIN s_b	PIETRAIN log a	PIETRAIN Impetus	LARGE WHITE Growth ratio b*	LARGE WHITE s_b	LARGE WHITE log a	LARGE WHITE Impetus	Sig. of diff.**
Shoulder & elbow									
Tensor fasciae antebrachii	0·905	0·061	-2·310	average	0·902	0·027	-2·216	low	NS
Triceps brachii (long head)	0·920	0·010	-1·195	low	0·945	0·012	-1·310	low	NS
Biceps brachii	0·917	0·020	-2·102	low	0·929	0·013	-2·125	low	NS
Total shoulder & elbow	0·917	0·009	-1·111	low	0·940	0·010	-1·206	low	NS
Elbow									
Brachialis	0·885	0·016	-1·807	low	0·880	0·012	-1·787	low	NS
Triceps brachii (lateral head)	0·850	0·018	-1·569	low	0·881	0·015	-1·639	low	NS
Triceps brachii (medial head)	0·869	0·034	-1·944	low	0·957	0·029	-2·204	average	NS
Anconeus	0·759	0·058	-2·068	low	0·849	0·042	-2·234	low	NS
Total elbow	0·855	0·014	-1·202	low	0·893	0·013	-1·299	low	NS
Total brachium	0·913	0·008	-0·605	low	0·953	0·009	-0·746	low	P < 0·005
ANTEBRACHIUM									
Extensor carpi radialis	0·862	0·013	-1·722	low	0·903	0·017	-1·849	low	NS
Ulnaris lateralis	0·802	0·031	-2·482	low	0·800	0·047	-2·355	low	NS
Extensor carpi obliquus	0·825	0·020	-2·646	low	0·796	0·061	-2·575	low	NS
Extensor digitorum communis	0·870	0·033	-2·181	low	0·882	0·029	-2·205	low	NS
Extensor digitorum lateralis	0·792	0·020	-2·098	low	0·833	0·062	-2·190	low	NS
Flexor carpi radialis	0·833	0·015	-2·355	low	0·922	0·028	-2·631	low	P < 0·01
Flexor carpi ulnaris	0·761	0·024	-2·456	low	0·898	0·038	-2·838	low	P < 0·01
Flexor digitorum superficialis & profundus	0·891	0·018	-1·659	low	0·869	0·075	-1·581	average	NS
Total antebrachium	0·862	0·011	-1·166	low	0·879	0·019	-1·199	low	NS
MANUS									
Mm. interossei etc.	0·749	0·081	-2·240	low	1·194	0·190	-3·722	average	P < 0·05
CUTANEUS	1·104	0·040	-1·948	high	1·119	0·046	-1·898	high	NS
DORSUM									
Longissimus thoracis	1·098	0·017	-1·581	high	1·049	0·016	-1·426	high	NS
Longissimus lumborum	1·160	0·028	-1·991	high	1·116	0·018	-1·847	high	NS
Total longissimus	1·120	0·016	-1·442	high	1·075	0·013	-1·299	high	P < 0·05

Table 14 (continued)

Muscle	PIETRAIN Growth ratio b*	s_b	log a	Impetus	LARGE WHITE Growth ratio b*	s_b	log a	Impetus	Sig. of diff.**
ABDOMEN									
Obliquus abdominis externus	1·029	0·026	-1·701	average	0·997	0·033	-2·046	average	NS
Rectus abdominus	1·049	0·021	-1·917	high	1·021	0·028	-1·843	average	NS
Obliquus abdominis internus	1·043	0·017	-2·104	high	1·224	0·062	-2·767	high	P < 0·01
Transversus abdominis	1·069	0·027	-2·056	high	1·013	0·034	-1·852	average	NS
Total abdomen	1·045	0·017	-1·333	high	1·060	0·020	-1·493	high	NS
THIGH									
Hip									
Ilio-psoas	1·073	0·031	-1·915	high	0·980	0·015	-1·573	average	P < 0·05
Tensor fasciae latae	1·059	0·014	-2·223	high	1·005	0·023	-2·094	average	P < 0·05
Gluteus superficialis	1·121	0·062	-2·215	average	1·113	0·028	-2·193	high	NS
Gluteus medius	0·997	0·028	-1·692	average	1·053	0·028	-1·968	average	NS
Gluteus accessorius	1·045	0·026	-2·230	average	1·021	0·027	-2·164	average	NS
Gluteus profundus	1·048	0·030	-2·548	average	1·062	0·062	-2·558	average	NS
Gracilis	1·026	0·013	-2·023	average	1·013	0·013	-2·014	average	NS
Sartorius	0·977	0·059	-2·816	average	0·946	0·047	-2·734	average	NS
Adductor	1·095	0·096	-2·059	average	1·043	0·043	-1·962	average	NS
Pectineus	1·018	0·019	-2·425	average	1·003	0·016	-2·379	average	NS
Quadratus femoris	0·929	0·043	-2·674	average	1·026	0·047	-3·094	average	NS
Obturator	1·128	0·061	-2·763	average	1·046	0·025	-2·381	average	NS
Total hip	1·041	0·010	-1·153	high	1·035	0·007	-1·142	high	NS
Hip and stifle									
Biceps femoris	1·067	0·015	-1·380	high	1·057	0·014	-1·365	high	NS
Semitendinosus	1·078	0·017	-1·943	high	1·036	0·019	-1·818	average	NS
Semimembranosus	1·073	0·029	-1·534	high	1·043	0·015	-1·470	high	NS
Rectus femoris	1·021	0·012	-1·738	average	0·987	0·013	-1·621	average	NS
Total hip and stifle	1·060	0·015	-0·932	high	1·035	0·008	-0·871	high	NS
Stifle									
Mm. vasti	1·045	0·014	-1·602	high	1·011	0·014	-1·504	average	NS
Popliteus	0·962	0·018	-2·454	low	0·890	0·025	-2·176	low	P < 0·05
Total stifle	1·039	0·014	-1·550	high	1·000	0·013	-1·433	average	NS
Total thigh	1·051	0·008	-0·664	high	1·030	0·006	-0·614	high	P ≏ 0·05

Table 14 (continued)

Muscle	PIETRAIN Growth ratio b*	s_b	log a	Impetus	LARGE WHITE Growth ratio b*	s_b	log a	Impetus	Sig. of diff.**
CRUS									
Tibialis cranialis	0·921	0·022	-2·448	low	0·925	0·018	-2·467	low	NS
Fibularis tertius & extensor digitorum longus	1·047	0·085	-2·605	average	0·984	0·012	-2·348	average	NS
Extensor digitorum lateralis	0·983	0·021	-2·709	average	0·961	0·022	-2·589	average	NS
Soleus and gastrocnemius	1·019	0·018	-1·706	average	1·020	0·019	-1·706	average	NS
Fibularis longus	0·942	0·034	-2·542	average	0·877	0·076	-2·277	average	NS
Flexor digitorum superficialis	0·966	0·035	-2·223	average	0·959	0·020	-2·246	average	NS
Flexor digitorum profundus	1·010	0·025	-2·114	average	1·060	0·070	-2·299	average	NS
Extensor digiti I longus	0·848	0·045	-3·129	low	0·821	0·051	-3·034	low	NS
Total crus	0·997	0·015	-1·310	average	0·987	0·011	-1·269	average	NS
PES									
Extensor digitorum brevis	0·807	0·024	-2·302	low	0·858	0·034	-2·444	low	$P < 0·05$
Mm. interossei etc.	1·009	0·056	-3·223	average	0·773	0·055	-2·250	low	$P < 0·01$

Muscles with the highest growth ratios are found in the femoral, lumbar and abdominal regions. Muscles with lower growth ratios occur in the distal hindlimb, and in the pectoral, brachial and neck regions. Muscles with the lowest growth ratios are found in the head and distal forelimb. The growth ratios obtained from regressions of muscles grouped according to their skeletal attachments form a similar pattern (Figs. 43 and 44).

Significant differences occur between the two breeds in the growth ratios of only 10 individual muscles (Table 14). However, the extremes of relative growth shown diagrammatically for the Pietrain in Figs. 41 and 43 are not as wide as shown for the Large White in Figs. 42 and 44. When 10 major muscle

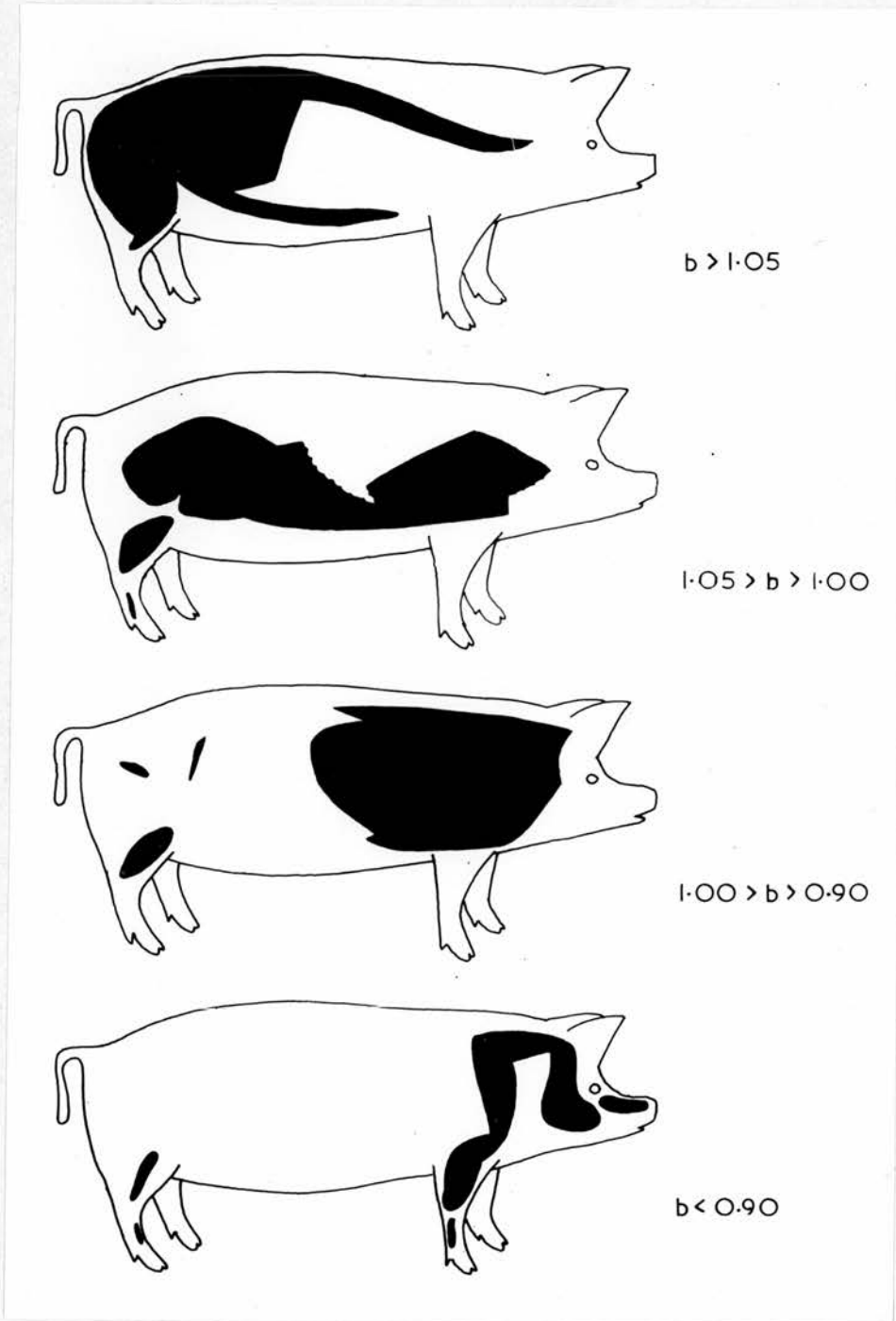


Fig. 41. Growth changes in muscle distribution of the female Pietrain pig between birth and 64 kg liveweight. Individual muscles are allocated to four groups according to their growth ratio b (Table 14), and their topographical locations are outlined.

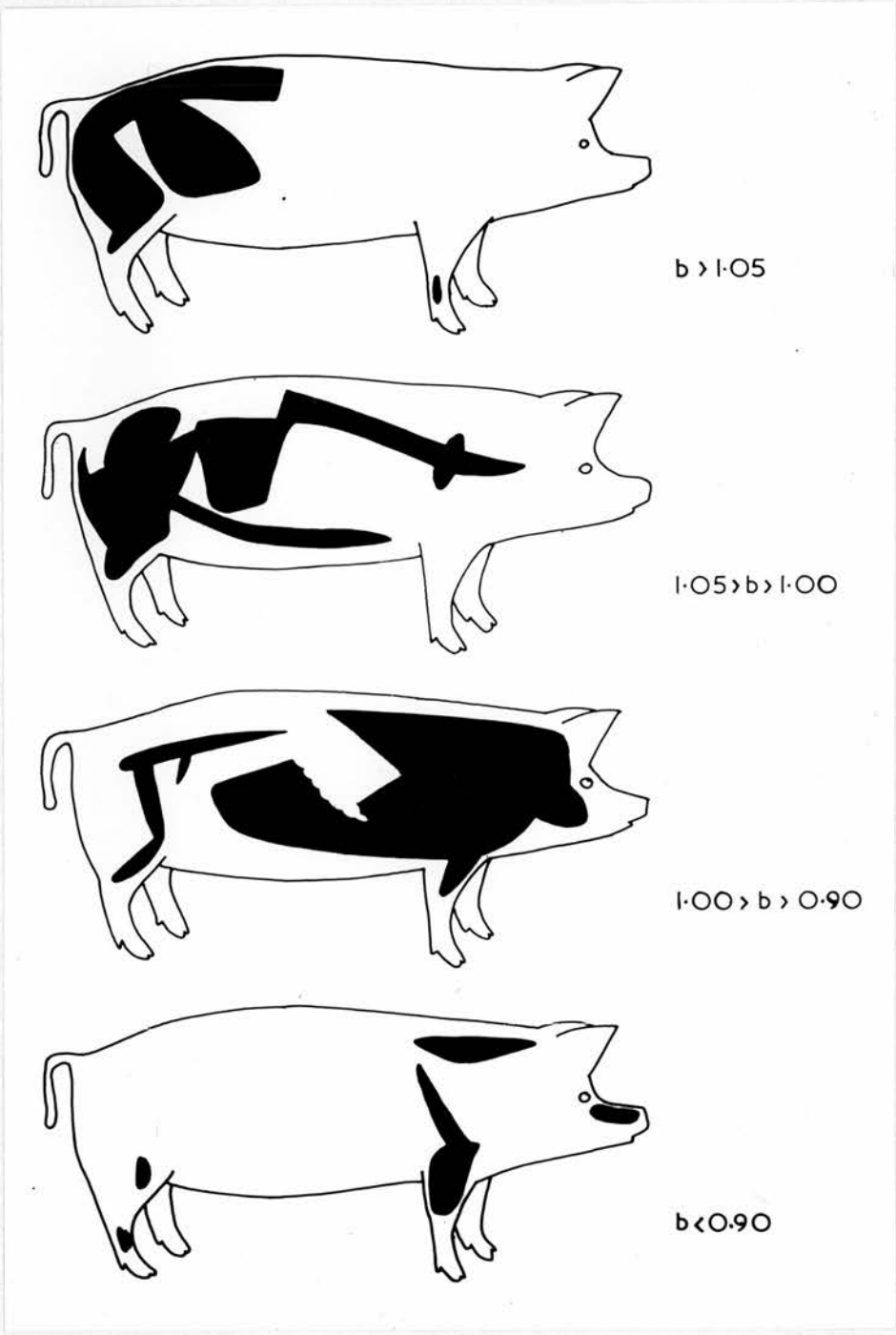
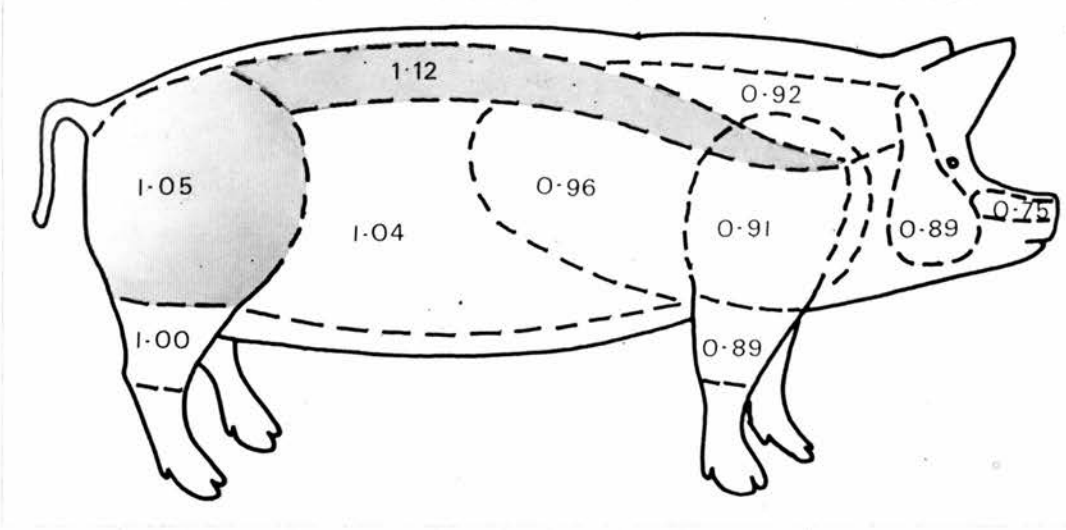
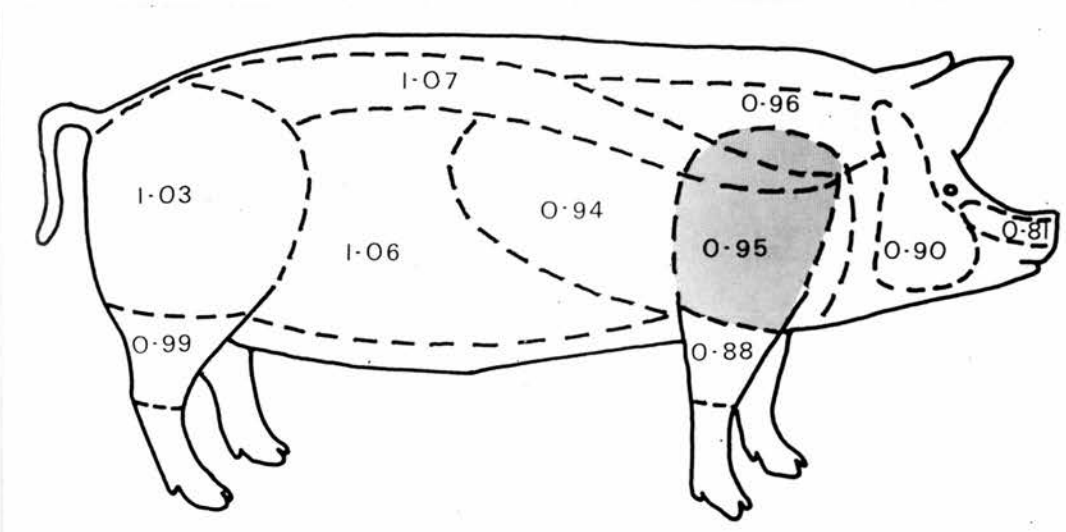


Fig. 42. Growth changes in the muscle distribution of the female Large White pig between birth and 64 kg liveweight. Individual muscles are allocated to four groups according to their growth ratio b (Table 14), and their topographical locations are outlined.



43



44

Figs. 43, 44. Growth of muscles, allocated to 10 groups according to their skeletal attachments, relative to the growth of total side muscle (Table 14) for Pietrain (Fig. 43) and Large White (Fig. 44) female pigs from birth to 64 kg liveweight. Muscle groups with a growth ratio significantly higher between breeds are indicated by shading.

groups are compared (Figs. 43, 44 and 45), growth ratios of the femoral muscles and m. longissimus are significantly higher for the Pietrain, and the growth ratio of muscles in the brachial region is significantly higher for the Large White.

In order to determine how these patterns of growth affect the muscle development of the whole animal, the weight of 10 muscle groups are compared between the two breeds in Table 10c and Fig. 45 at EBWs of 2 and 60 kg. At birth, the weights of all the muscle groups are significantly higher for the Pietrain. There is no topographical pattern in the ratios of these weights, and therefore there is no suggestion that the higher TSM weight of the Pietrain at birth (Table 10b) is due to relatively high muscle weights in any particular region. In pigs of 60 kg EBW however, only abdominal and femoral muscles and m. longissimus are significantly heavier for the Pietrain. The ratios of the weights of different muscle groups show a significantly declining growth gradient similar to that established for growth ratios of these different muscles of each breed. The significantly higher TSM weight in the 60 kg EBW Pietrain is due to heavier high impetus muscles, rather than a higher overall muscle weight.

When the weight of cardiac muscle is calculated using a regression on TSM (Table 10c) rather than EBW (Table 10a), similar values are obtained. Because the variance of these values is less than for the regression on EBW, the difference in heart weights between the two breeds at 60 kg EBW is now significant. At both EBWs, the weight of cardiac muscle as a percentage of total muscle is significantly higher for the Large White pig (Table 15).

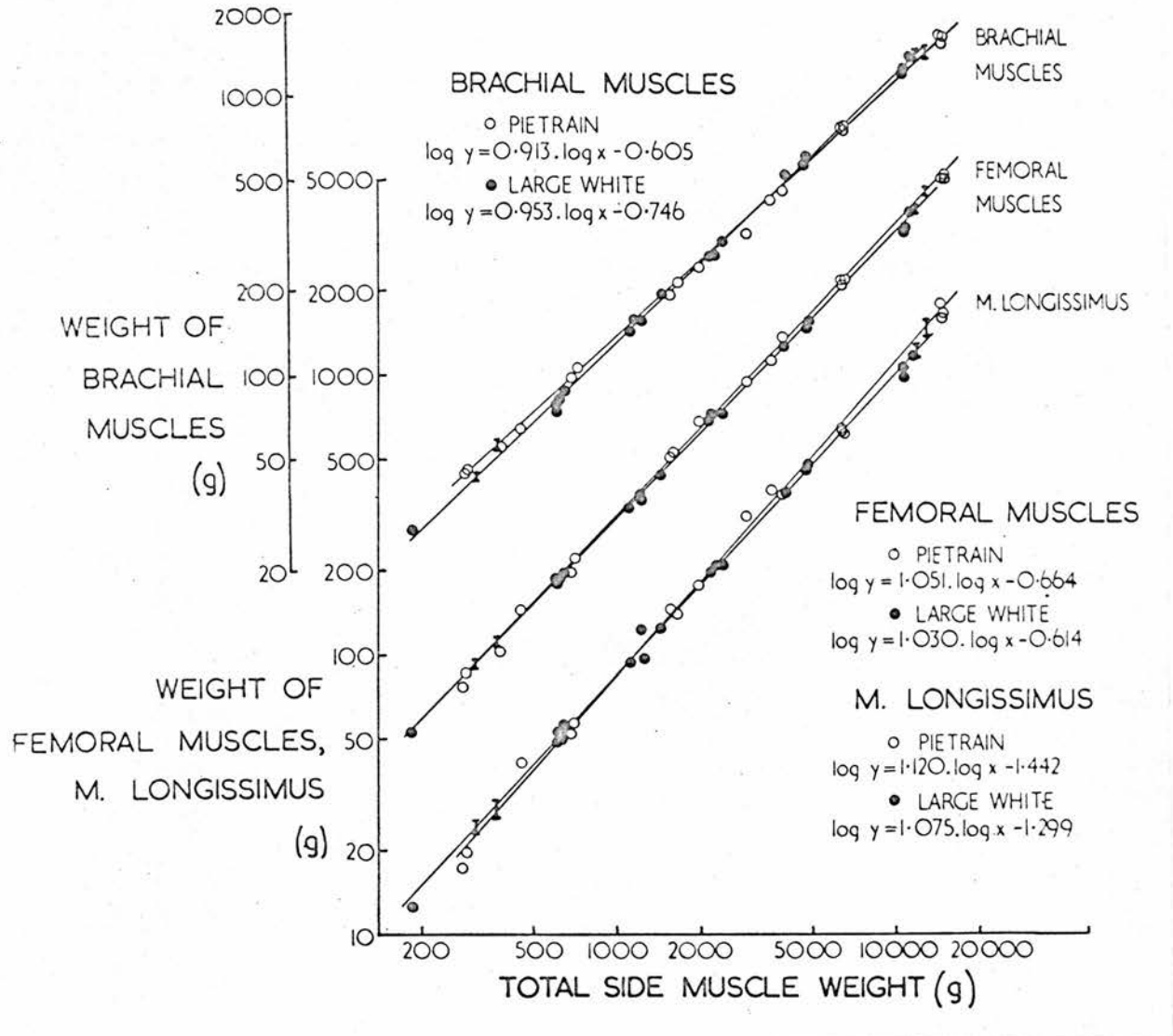


Fig. 45. Allometric equations comparing the growth of femoral and brachial muscle groups and m. longissimus, relative to total side muscle, between Pietrain and Large White female pigs.

I 95% confidence limits for muscle weights in pigs of 2 and 60 kg empty body weight.

Table 15. Comparison of weight ratios of body components between Pietrain and Large White female pigs of 2 and 60 kg empty body weight.

Weight ratio	Empty body weight 2 kg: Pietrain	Large White	Significance*	Empty body weight 60 kg: Pietrain	Large White	Significance*
$\frac{\text{Heart} \times 100}{2 \times \text{TSM}}$	1·81	2·41	$P < 0{\cdot}001$	0·88	1·12	$P < 0{\cdot}001$
$\frac{\text{TSM}}{\text{TSB}}$	2·78	2·34	$P < 0{\cdot}001$	5·76	3·80	$P < 0{\cdot}001$
$\frac{\text{Femoral muscles}}{\text{Femur}}$	9·65	7·93	$P < 0{\cdot}001$	20·78	12·97	$P < 0{\cdot}001$
$\frac{\text{Brachial muscles}}{\text{Humerus}}$	5·28	3·79	$P < 0{\cdot}001$	7·49	5·83	$P < 0{\cdot}001$

*Significance of the difference between breeds tested at the 5% level by the test quotient t.

The development of a higher proportion of skeletal muscle in the Pietrain has not been accompanied by a comparable development of circulatory capacity.

3.3.4 Growth of bone

The relative growth of the individual bones and bone groups that were dissected are compared between the two breeds, and between different body regions, in Table 16. No growth ratios are significantly different between breeds. Within each breed there is, however, a consistent pattern of growth. Growth in the axial skeleton is greater in the caudal than in the cranial region. The difference is significant ($P < 0{\cdot}005$) only for the Pietrain.

Table 16. Regression equations comparing the growth of bones relative to total side bone in 18 Pietrain and 18 Large White female pigs from birth to 64 kg liveweight.

Weight of y = a.(total side bone)^b

Bone y.	PIETRAIN Growth ratio b*	s_b	log a	Impetus	LARGE WHITE Growth ratio b*	s_b	log a	Impetus	Sig. of diff.**
Cranial axial skeleton (excluding skull)	0·988 BC	0·017	-0·410	average	0·994 B	0·020	-0·426	average	NS
Scapula	1·072 A	0·018	-1·517	high	1·068 A	0·023	-1·511	high	NS
Humerus	1·017 B	0·013	-1·137	average	0·961 BC	0·037	-0·989	average	NS
Radius & ulna	0·963 CD	0·014	-1·100	low	0·975 B	0·016	-1·117	average	NS
Carpus & metacarpus	0·923 D	0·021	-1·167	low	0·911 C	0·017	-1·073	low	NS
Caudal axial skeleton (excluding caudal vertebrae)	1·078 A	0·020	-1·041	high	1·049 A	0·039	-0·947	average	NS
Femur	1·043 A	0·017	-1·156	high	1·032 AB	0·020	-1·137	average	NS
Patella	1·080 AB	0·052	-2·488	average	1·099 A	0·040	-2·557	high	NS
Tibia & fibula	0·980 B	0·019	-1·101	average	0·986 BC	0·020	-1·120	average	NS
Tarsus & metatarsus	0·897 C	0·021	-0·828	low	0·930 C	0·021	-0·909	low	NS

*Regression coefficient b, with standard deviation s_b.
Values of b within each limb of each breed not followed by the same letter are significantly ($P < 0·05$) different.
**Significance of the difference in b between breeds, tested at the 5% level by the test quotient t; NS = not significant.

In the forelimb from the scapula distally, and in the hindlimb from the femur distally, there is a gradient of significantly declining growth. However, the scapula has a higher growth ratio than the cranial axial skeleton, and the femur and patella are not significantly different in growth from the caudal axial skeleton. These results are shown graphically in Fig. 46. In the pig, the growth gradients for bone parallel those demonstrated for muscle.

The weights of individual bones in pigs of 2 and 60 kg EBW are shown in Table 10d. Summation of the calculated weights of bones for each breed at

both EBW weights gives a value of TSB very close to the value used in each of the regression equations (Table 10b). This is evidence that the allometric equations adequately describe bone growth in the animals studied. At 2 kg EBW, bone weights are significantly different between the two breeds only for the carpus and metacarpus. At 60 kg EBW, the bones are all significantly heavier in the Large White. The ratios of these weights show no definite pattern of topographical variation. In particular, for the pigs of 60 kg the ratios for the bones in the caudal axial and femoral regions are not significantly higher than the ratios for bones in other regions. The genetic effect enhancing the growth of high impetus muscles in the Pietrain does not influence the growth of high impetus bones. Thus although the muscle:bone ratio is higher for the Pietrain at both EBWs in the entire half carcass and in both the femoral and brachial regions (Table 15), the greatest difference in this ratio is seen in the femoral region of the 60 kg EBW pigs.

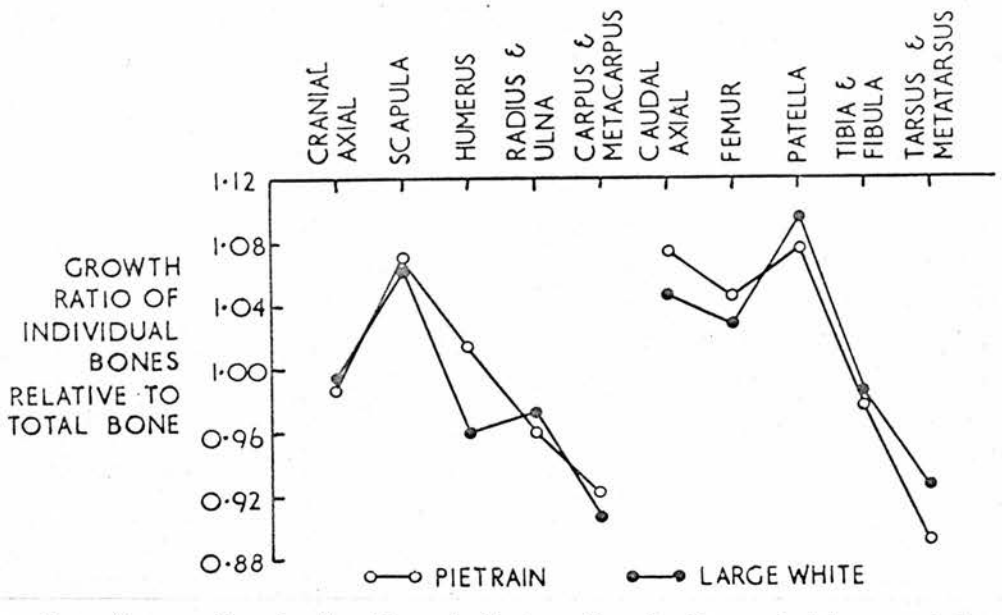


Fig. 46. Growth gradients in the skeleton (excluding skull, caudal vertebrae and digits) of Pietrain and Large White female pigs between birth and 64 kg liveweight.

3.3.5 Growth of m. longissimus

3.3.5.1 The relation between longissimus TSA and weight of total muscle

Allometric regressions of weights of TSM and longissimus on width x depth of longissimus have been calculated for both breeds. All these regressions have high residual variances; there are no significant differences in growth ratios between the two breeds. The weights of TSM and longissimus, and their variance, have been calculated for a value of longissimus width x depth at the top of the range studied (50 cm^2). These weights, and their 95% confidence limits, are compared in Table 17. The estimate of both longissimus weight and TSM weight is lower for the Pietrain. Although the differences are not significant, the finding suggests that a measurement of TSA of longissimus will overestimate the total muscle weight of the Pietrain unless the existence of a special relationship between total muscle weight and longissimus TSA for this breed is appreciated. The use of longissimus TSA to compare directly the proportion of muscle in different breeds of pigs (Allen, Forrest, Chapman, First, Brag & Briskey, 1966; Topel, Merkel & Wismer-Pederson, 1967; Bichard, 1968) is therefore questionable.

Table 17. Weights of longissimus and TSM compared between Large White and Pietrain pigs with a width x depth value for m. longissimus of 50 cm^2.

	Muscle weight (g)			
	PIETRAIN		LARGE WHITE	
	Mean	95% limits	Mean	95% limits
M. longissimus	1720	1470 - 2010	1840	1460 - 2310
TSM	15000	13200 - 17000	17600	14300 - 21800

3.3.5.2 The relation between carcass weight and TSA of m. longissimus

Measurements of width x depth of m. longissimus are shown in a double logarithmic plot against carcass weight in Fig. 47. The growth ratios are not significantly different between the two breeds. However, since the 95% confidence limits do not overlap, the values of width x depth are higher for the Pietrain at any given carcass weight over the whole growth range.

3.3.5.3 The relation between fibre TSA and total TSA of m. longissimus

Double logarithmic regressions of mean fibre TSA on width x depth are plotted in Fig. 48. When one Large White pig of 2 days of age is eliminated, the regression lines for each breed are not significantly different. The regression coefficient for the Large White is significantly different from 1; this suggestion that mean fibre TSA is not directly proportional to width x depth apparently contradicts the previous findings (paragraph 2.3.2.2; Fig. 16) where the TSA of longissimus was used in a similar regression for the same Large White pigs. However, when the growth of TSA and width x depth for the Large White longissimus are compared, it is found that the TSA is proportional to the 0·898 (SD = 0·024) power of width x depth rather than being in direct proportion. This indicates that a change in the shape of the transverse section of the muscle occurs with growth, from an angular form to a more rounded elliptical form.

Accepting the earlier results for the Large White, since the lines for the double logarithmic regression of mean fibre TSA on width x depth for both breeds are nearly parallel (Fig. 48), the number of fibres in m. longissimus of the Pietrain is also constant during the period of postnatal growth studied. The intercept of the regression lines is the same; therefore the

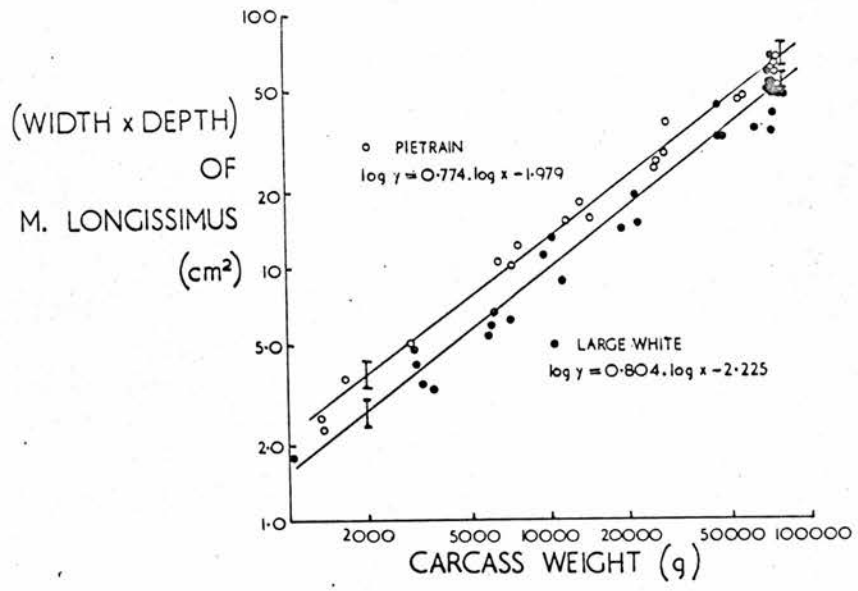


Fig. 47. Changes in TSA of m. longissimus (estimated by the value of width x depth of the muscle) with increasing carcass weight, in Pietrain and Large White female pigs.

I 95% confidence limits at carcass weights of 2 and 60 kg.

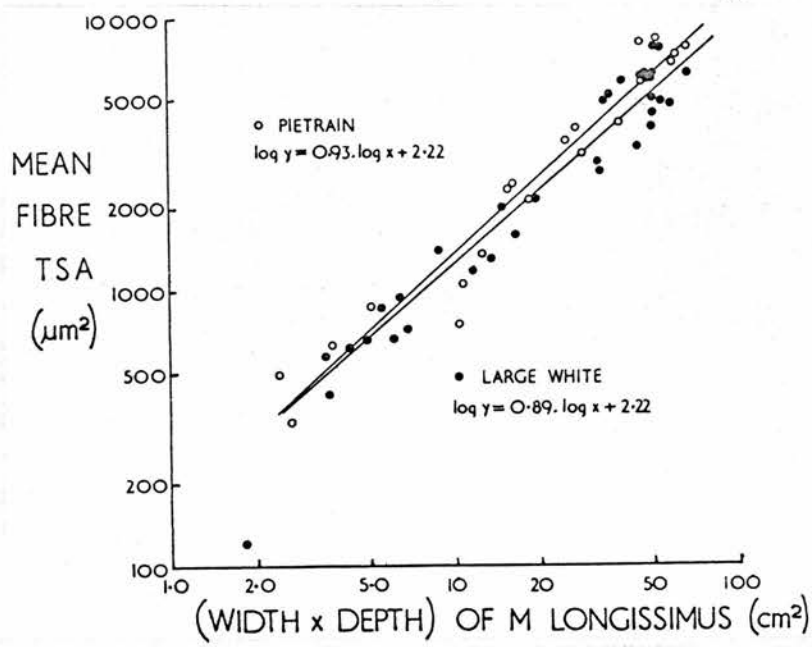


Fig. 48. Changes in the mean fibre TSA of m. longissimus with increasing TSA of the muscle (estimated by the value of width x depth) in Pietrain and Large White female pigs.

number of fibres at any given width x depth value is the same for both breeds, and the greater width x depth of m. longissimus of the Pietrain at the same carcass weight (Fig. 47) is due to hypertrophy of a similar number of component fibres. This conclusion depends, however, on the assumption that there is no difference between breeds in the extent to which the muscle contracts when the samples are excised.

3.3.5.4 Growth changes in the proportion of myosin ATPase low fibres in m. longissimus of Pietrain and Large White pigs

With increasing liveweight, there is an increase in the proportion of myosin ATPase low fibres in m. longissimus of both breeds (Fig. 49). There is a large variation between samples, and no suggestion of a difference between breeds.

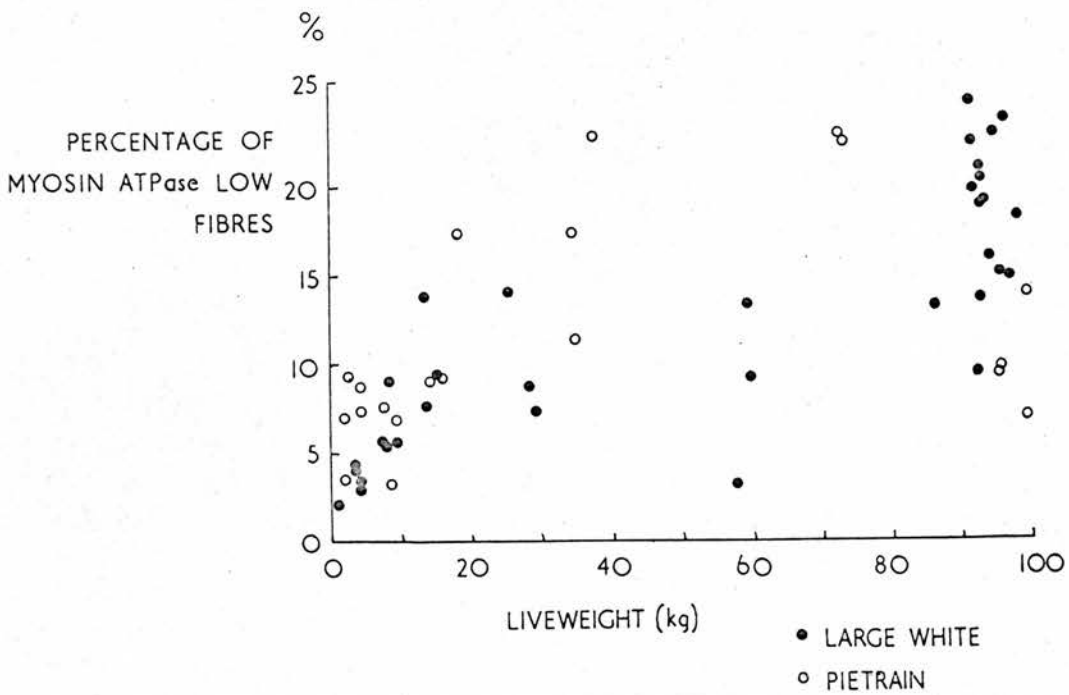


Fig. 49. Growth changes in the proportions of histochemical fibre types of m. longissimus of Pietrain and Large White pigs, as determined by the myosin ATPase reaction.

3.4 DISCUSSION

3.4.1 Factors affecting the proportions of muscle, bone and fat in meat producing animals

The proportions of muscle, bone and fat in the carcass would be expected to bear some relation to the function of these tissues in the living animal. The tissues that support the body (including muscle and bone), the muscle that provides an accelerating force, and the bone that applies this force at useful locations, must adapt during growth if their effect on the body is to be constant. It is expected that either the mass or the mechanical properties of these tissues will be related in some way to body size. Other functions of bone, muscle and fat are not, however, linked to the size of the animal. For instance, muscle, bone and fat are all reservoirs of metabolites necessary to maintain nutritional balance; muscle for protein metabolism, bone for calcium metabolism, and fat for energy metabolism. The metabolic state of the animal could therefore override the effect of body size on tissue proportions. This is especially likely in the case of the mechanically inert tissue fat.

The relevance of tissue proportions to meat production has encouraged studies that examine the above proposals. Tulloh (1964) reanalyses allometrically the growth data obtained by several workers. The within species variation in muscle and bone weights, but not fat weights, of cattle, sheep and pigs is explained almost entirely by the effect of body size. However, the growth of muscle relative to body weight in sheep subject to nutritional extremes is shown by Boccard & Dumont (1970) to be lower when the rate of body growth is higher. In a similar experiment, Boccard, Le Guelte

& Arnoux (1964) show that this trend is reversed for fat growth. By reanalysing the data from the dissection studies of McMeekan (1940a, b, c) on Large White pigs and Pálsson & Vergés (1952) on cross-bred lambs, Elsley, McDonald & Fowler (1964) conclude that nutritional extremes have little effect on the weight of muscle or bone at the same total muscle plus bone weight. It is apparent that within a species, muscle and bone bear a definite relation to body size, but nutritional extremes that produce variable fat deposition can alter this relationship.

The predictable effect of body size on the proportions of muscle and bone in animals with similar genotype and nutritional status cannot be extended to animals of different species, or even of different breeds. The allometric equations derived by Tulloh (1964) enable the calculation of hypothetical muscle and bone weights for sheep and pigs of 2 and 60 kg EBW, and cattle of 60 and 800 kg EBW (Table 18). At all weights in the three species, there is comparatively little variation in muscle weight as a percentage of EBW. However, the proportion of bone is similar among sheep and pigs of 2 kg and cattle of 60 kg, and is also similar (but much lower than for neonates) among sheep and pigs of 60 kg and cattle of 800 kg. Thus there is a similarity in muscle:bone ratios in the neonates of the three species, and in the adults; this suggests that between species, maturity has a greater effect on the muscle:bone ratio than body size. Genotype is therefore an important source of variation in this parameter.

Since there are large differences in muscle:bone ratios between species at the same body weight, it is likely that there are smaller, though significant differences in the muscle:bone ratio between breeds.

Table 18. Proportions of muscle and bone in cattle, sheep and pigs at two stages of growth, calculated from the allometric equations of Tulloh (1964) relating tissue weight to empty body weight.

Weight ratio \ Empty body weight	2 kg		60 kg			800 kg
	Sheep	Pig	Ox	Sheep	Pig	Ox
$\frac{\text{Muscle weight}}{\text{EBW}} \times 100$	28·9	34·5	45·5	30·0	33·5	38·2
$\frac{\text{Bone weight}}{\text{EBW}} \times 100$	16·2	18·4	18·7	6·3	9·4	8·4
Muscle:bone ratio	1·78	1·87	2·44	4·76	3·54	4·57

Berg & Butterfield (1966) compare the muscle:bone ratios of Hereford, Brahman cross, Angus and unimproved North-central Australian Shorthorn steers after adjusting tissue and carcass weights to a common level by covariance analysis. They demonstrate significant differences in the muscle:bone ratios and in the growth of muscle relative to muscle plus bone. Dumont & Boccard (1967) find muscle:bone ratios of 4·2, 4·2, 4·7 and 5·8 in bulls of 200 kg muscle weight of the Fresian, Normandy, Charolais and Limousin breeds respectively. Mukhoty & Berg (1971) study Hereford, Jersey and Holstein cattle, and six types of crosses that involve the Hereford, Charolais, Angus, Galloway, Shorthorn and Brown Swiss breeds. The growth of muscle and bone, relative to the growth of total muscle plus bone, is similar for all the breeds, but when the weights of tissues are compared at the same weight of muscle plus

bone, there are significant differences between breeds. In particular, for the purebred bulls the muscle:bone ratio is highest for the Jersey (5·55), intermediate for the Hereford (5·46) and lowest for the Holstein (4·12), and for the purebred steers the ratio is higher for the Hereford (4·99) than for the Holstein (4·01). Richmond and Berg (1971a) compare the muscle:bone ratios of Yorkshire pigs with Duroc x Yorkshire and Hampshire x Yorkshire crosses at 68, 91 and 114 kg liveweights. Possibly because these breeds do not represent extremes of pig development, differences are not significant between breeds. Dumont, Schmitt & Roy (1969) compare the proportions of muscle and bone of 5 Pietrain and 5 Large White pigs with mean half-carcass weights of 32·2 and 35·2 respectively. Mean weights are higher for TSM in the Pietrain, and for TSB in the Large White. The present study confirms this result (Table 10b).

Within and between species, therefore, genotype influences the proportion of muscle and bone. Breed differences could represent either adaptions to differing functional demands on the tissues, differences in the relationship between the weight of a tissue and its functional capacity, or, for bone in particular, differences in the degree of maturity at the same body weight. The relationship between bone weight and body weight depends on whether animals are compared between mature members of different species, or between individuals of the same species during growth. Between adult examples of various species, bone weight is proportional to the 1·13 power of body weight (Kayer & Heusner, 1964), and during growth of sheep, cattle and pigs to the 0·72, 0·70 and 0·80 power of body weight respectively (Tulloh, 1964). The process of maturation of bone is not merely a mechanical adaption to an increase in body size. Since the pigs of the present study have, with

maturity, a higher proportion of muscle and a lower proportion of bone, the Pietrain is, in this respect at least, more mature at a given body weight. The apparent failure to change the muscle:bone ratio of the Large White pig during more than 30 years of genetic improvement indicates that current selection practices are having little effect on this parameter. Should it be considered desirable, however, there is phenotypic variation in the tissue proportions of modern breeds of meat producing animals that invites exploitation.

3.4.2 The effect of body size on the distribution of muscle and bone

D'Arcy Thompson (1917, 1942) shows that evolutionary changes in form can be expressed as transformations of a coordinate system. Huxley (1932) applies this concept to growth, using allometry to describe these transformations as gradients of growth along body axes, to demonstrate, for example, that the postnatal growth of the bones in the forelimbs of the sheep, relative to the growth of the vertebrae, increases proximally. Most investigators have, however, studied the regional growth of bone and muscle of animals by the use of arithmetic, rather than logarithmic, ratios.

McMeekan (1940a) compares the bone weights, relative to the weight of each bone at birth, of entire and castrated male pigs from birth (1·3 kg) to 100 kg empty body weight. The ratio increases caudally in the axial skeleton, and proximally in both the fore- and hindlimbs. Individual muscles were not dissected, but the growth gradient of the muscle tissue from 'joints' of the carcass corresponds to that of the bones of the corresponding regions. McMeekan (1943) and Pálsson (1955) use this general pattern to describe the

regional development of meat animals. Other studies indicate, however, that growth changes in the distribution of muscle and bone in the pig and other meat animals are more complex.

Cuthbertson & Pomeroy (1962) study bone growth in female and castrated male pigs of carcass weights of 50, 68 and 92 kg. Between 50 and 68 kg carcass weight, the percentage increase in the weight of parts of the axial skeleton is greater caudally, but this pattern is reversed between 68 and 92 kg carcass weight. The pattern of bone growth in the limbs of these pigs between 50 and 68 kg carcass weight is similar to that found by McMeekan (1940a), but during subsequent growth no clear gradient is demonstrated. Wallace (1948), using female and castrated male lambs, shows that although the growth of bone and muscle tissues contained in 'joints' of the carcass increases caudally in lambs from birth (6 kg) to 28 kg liveweight, there is no definite gradient during subsequent growth stages up to 61 kg liveweight. Pálsson & Vergés (1952) dissected the individual bones of female and castrated male sheep of 26 and 81 kg liveweight, and compared the weights of bones expressed as a ratio of the weight of the total weight of metacarpals and metatarsals with the value of this ratio at birth (4·1 kg liveweight). Although there is an overall pattern of growth similar to that found by McMeekan (1940a) for the pig, growth of forelimb bones as a whole exceeds that of hindlimb bones, and the growth of the ribs exceeds that of all other bones measured. In female, male and castrated male Merino and Dorset Horn crossbred lambs between 13·5 and 35·5 kg liveweight, Seebeck (1968) finds that as total muscle weight increases, the proportion of the total muscle in 'joints' described as 'neck' and 'loin and flank' increases, while that in the 'thorax' and 'leg' decreases

and that in the 'shoulder' does not change. There is a decrease in the bone of the 'leg' relative to total bone, but no other significant changes in bone distribution are observed during this stage of growth. Luitingh (1962) studies the growth of undissected 'joints' of steer carcasses. The 'loin' is the fastest growing 'joint' in steers reared from 8 months of age, but during the 'fattening' of cattle from both 20 and 32 months of age, the growth of 'neck' and 'thorax' is greater than that of other regions. No growth gradients are evident in these cattle. Butterfield & Berg (1966b) analyse allometrically the data obtained from the dissection of individual muscles from 92 calves and steers. Muscles grouped proximally in the hindlimb have a higher growth ratio than distal muscles, in calves up to 84 days of age. However, for these animals there is no pattern of muscle growth apparent either during subsequent development of the limbs or during any stage of the development of the trunk.

It is apparent from these previous observations that the distribution of muscle and bone is affected by body size alone only in the early stage of postnatal growth. The animals selected for these studies represent different growth ranges, and in most instances, more than one sex. The data obtained do not indicate what influences other than body size affect muscle and bone distribution.

The present study confirms allometrically that postnatal growth of bone and muscle in the female pig from birth to 64 kg liveweight is satisfactorily described in terms of increasing craniocaudal and ventrodorsal gradients. The change in shape is simulated by the Cartesian transformation of Fig. 50. For this to receive an explanation in terms of the hypothesis introducing

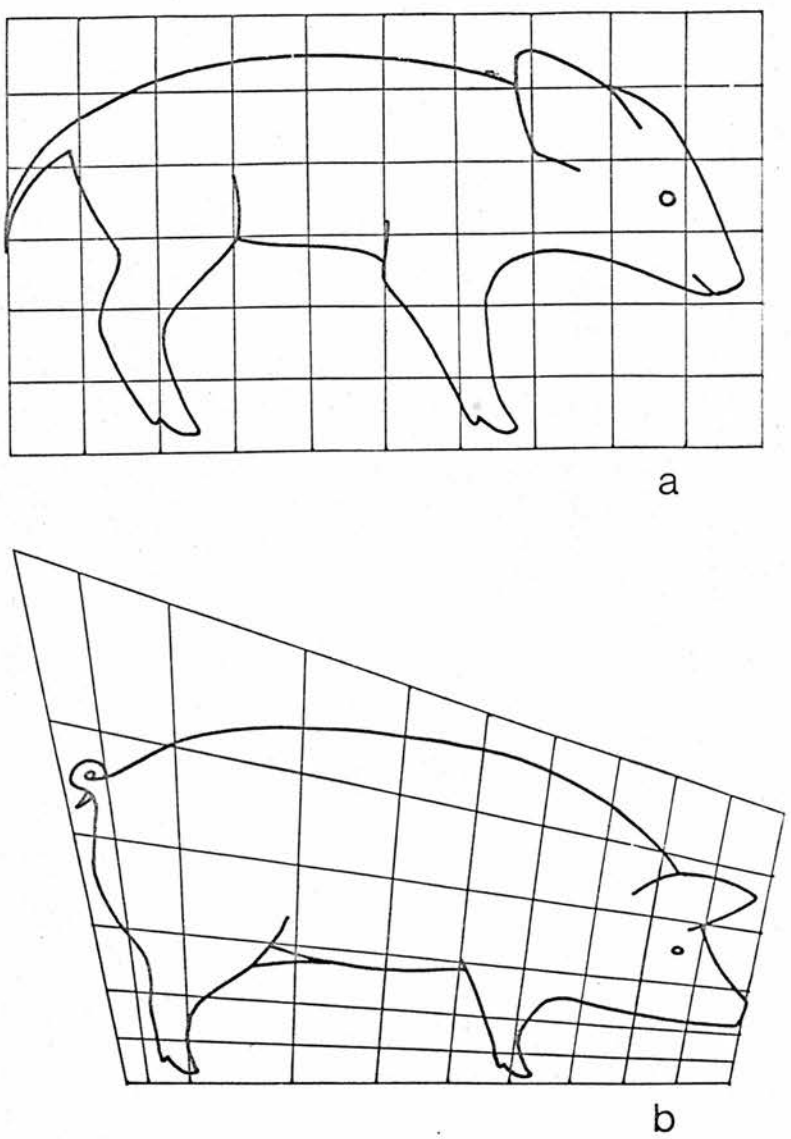


Fig. 50. Cartesian transformation of body shape in the pig. The outlines are scaled to the same body length. Fig. 50a is adapted from a drawing by Marrable (1971) of a male pig fetus of about 109 days gestational age. Fig. 50b is adapted from a photograph published by Dehaye (1962) of a Pietrain pig described as a "well developed young sow".

Part 2 of this thesis (paragraph 3.1.1), the ability of a muscle to assist propulsive acceleration of an animal should generally correspond to its growth impetus. It is also expected that the growth of the bones that these muscles act on is related to muscle growth. The present findings support this hypothesis. M. longissimus extends the vertebral column and the extensors of the hip provide a propulsive thrust to the hindlimb; the high growth impetus of these muscles would enhance the propulsive acceleration of the larger animal. The muscles of the crus and antebrachium, which are unlikely to play such a role, have a low growth impetus. However, the contribution of such muscles as m. serratus ventralis and the muscles of the abdomen to propulsive effort is not as obvious. Functional and histochemical investigations are necessary to determine precisely to what extent the harmonic growth patterns of the pig are related to the functional demands of an increase in body size.

3.4.3 The effect of breed on the distribution of muscle

The carcass dissections made by Butterfield (1964b) suggest that the cattle commonly used for beef production in Australia do not differ significantly in the distribution of their muscle tissue. Berg & Mukhoty (1970) obtained similar results using the dairy and beef purebred and crossbred cattle common in Alberta. Richmond & Berg (1971b) find significant differences only in muscles of the spinal group when the weights of muscle groups are compared between Yorkshire, Duroc x Yorkshire and Hampshire x Yorkshire pigs. These breeds of cattle and pigs do not, however, include the most extreme types of animal available for study today, and therefore do not adequately answer the

question of whether breeders are able to alter the muscle distribution of meat producing animals.

There is little evidence available to suggest that breeds of meat animals do vary in their muscle distribution. Seebeck (1968) demonstrates that Merino lambs have 15·7% more muscle in the neck region and 6·2% less muscle in the thoracic region than Dorset Horn crossbred lambs. Breed differences in the other 'joints' dissected in this study are not significant.

The data obtained by total muscle dissection of 5 Pietrain and 5 Large White carcasses by Dumont, Schmitt & Roy (1969) were not analysed statistically. However the weights of individual muscles and muscle groups, expressed as a ratio of TSM, suggest a greater development of the Pietrain in the lumbar and hindlimb regions, and of the Large White in the neck, shoulders and forelimb. This observation has been confirmed by the present study. Although limited evidence suggests that even the condition of 'muscular hypertrophy' of cattle occurs by the increased development of muscles in all regions of the carcass (Butterfield, 1966; MacKellar, 1968), the possibility of improving the muscle distribution of meat animals by genetic means is worthy of further investigation.

The present study indicates that the Pietrain has a more pronounced gradient of muscle growth than the Large White at the same body weight (Table 10c). This effect is sufficient to cause the greater muscle development of the Pietrain. As already shown for tissue proportions, therefore, the Pietrain is more 'mature' at lower body weights than the Large White.

3.4.4 Other factors affecting muscle distribution

Huxley (1932) provides many examples of striking allometric relationships between organs apparently totally unconcerned with support or locomotion, and body size. Thus the growth rate of the antlers of deer, the heads of neuter ants and the secondary sexual appendages of male beetles and mites, as a ratio of the growth of the whole body, is constant and greater than 1. The sex dependency of the growth of these organs suggests an analogous growth of sex dependent muscles such as the muscles of the head and neck of the male guinea-pig (Kochakian, Tillotson & Austin, 1957), the m. levator ani of the male rat (Wainman & Shipounoff, 1941) and the cervical muscles of the bull (Berg & Mukhoty, 1970). The demonstration of a receptor site for steroids in an androgen dependent muscle (Jung & Baulieu, 1972) supports the concept that these muscles become well-developed due to the primary growth stimulus of anabolic hormones rather than to the secondary effect of exercise hypertrophy due to sexual activity.

Butterfield & Berg (1966c) demonstrate that in calves, the plane of nutrition has no effect on the growth of high impetus muscles relative to total body weight. Extremes of energy levels in the rations fed to pigs from 23 kg liveweight had no significant effect on muscle distribution at 68, 91 or 114 kg liveweight (Richmond & Berg, 1971b). Skjervold, Standal & Bruflot (1963) exercised the hindlimb muscles of growing pigs by compelling them to feed twice daily with all their weight on their hind limbs. This treatment had no discernible effect on carcass weight or the weights of 7 hip muscles. If the extremes of nutrition and exercise likely to be encountered by meat producing animals do not influence the distribution of muscle, only two

mechanisms controlling differential growth of muscle within a breed need be recognised:

(1) the effect of body size, suggested here as the adaptive ability of an animal to develop preferentially certain muscles best suited to accelerate a growing body mass, and

(2) the growth stimulatory effect of anabolic hormones to develop muscles as secondary sex characteristics. This effect is presumably small in the immediate postnatal period, but with increasing age would become dominant as the growth rate declines and the animal becomes sexually mature.

The effect of anabolic steroids should therefore be considered when the allometric growth of individual muscles or muscle groups is found to be inconstant. Butterfield & Berg (1966a) provide growth ratios, but not complete allometric equations, for individual bovine muscles, based on total muscle dissections of 92 steers of a variety of breeds and crosses. Only for 21 steers aged up to 84 days, the breed of which is not stated, is the pattern of muscle growth comparable with that of the present study; during subsequent growth many muscles do not maintain a constant allometric relationship with total muscle. It is not apparent whether this is a characteristic of bovine muscles, whether it is due to the variety of breeds used, or, since information on the age of castration is not provided, whether it is due to a variable sex effect. The possible effect of anabolic steroids on muscle growth has also not been eliminated from the growth data of individual muscles of 20 ewes, 36 wethers and 26 rams of the Merino breed provided by Lohse, Moss & Butterfield (1971). These muscles also show biphasic growth patterns.

The present study has used female pigs of up to 6 months of age. The growth of all muscles, relative to the growth of total muscle, is monophasic. Therefore the changes in muscle distribution in these pigs are due mainly to the effect of increasing body size.

3.4.5 Characterisation of the Pietrain pig

The genetic origin of the Pietrain breed is unknown. A historical account of the development of the breed is given by Willems (1960). Pietrain pigs appeared about the year 1920 in the Jodoigne-Hannut region of the Belgian province of Brabant, and took their name from the small farming village of Piétrain in this locality. Since the breed was maintained by producers keeping only a few sows, there are no records of the circumstances under which it arose. It is apparent, however, that the tissue proportions of the Pietrain breed differ from those of breeds that would have been available as its progenitors, and that these proportions evolved without the benefit of the intense selection practices necessary to produce relatively small changes in other breeds. Although the breed's capacity for lean meat production aroused considerable interest in Europe during the 1950's and early 1960's (Kroes, 1960; Camerlynck, 1962; Van Snick & Camerlynck, 1966), disadvantages have been characterised in Europe, and in Britain following importation for experimental purposes in 1964. In particular, the breed is susceptible to the development of PSE muscles following slaughter (MacDougall & Disney, 1967; Charpentier, 1968; Lister, Scopes & Bendall, 1969; Lister, 1971; Lister & Ratcliff, 1971; Lean, Curran, Duckworth & Holmes, 1972).

Rühl (1971) compares the heart weights of pigs of the German Landrace, Pietrain, German Pasture, Mangalitza and Göttingen Miniature breeds in relation to an estimate of the weight of their musculature. The comparison is not made at the same liveweight, but since the cardiac muscle:skeletal muscle ratio is lower in the Pietrain at 88.1 kg than the Large White at 102 kg, the comparison of these two breeds agrees with the present findings. Thielscher (1966) finds that whereas the heart weight of German Landrace pigs is increased by exercise on a treadmill for 40 minutes daily during growth from 25 to 110 kg liveweight, there is no significant increase in the heart weight of Pietrains under the same conditions. Thielsher's study also provides evidence from electrocardiograph measurements that the Pietrain heart cannot adapt adequately to increased circulatory demands. The present study shows that the cardiac muscle:skeletal muscle ratio of the pig decreases with increasing body size, and that the Pietrain has a higher proportion of muscle (Table 10b) and a lower heart weight (Table 10c) than the Large White pig at the same liveweight. As already concluded for tissue proportions and muscle distribution, the differences in cardiac muscle: skeletal muscle ratio between the two breeds can be expressed as a greater maturity at the same body weight for the Pietrain. Present evidence suggests that in the pig, circulatory insufficiency is a problem inherent to selection for a higher lean body mass.

The present study shows that the muscularity of the Pietrain is the result of the greater development of high impetus muscles. Muscles such as longissimus and semimembranosus can therefore demonstrate the morphological, mechanical and metabolic differences contributing to or resulting from this muscularity. Staun (1963) reports that the muscle fibres of m. longissimus

of the Pietrain are larger than those of the Danish Landrace, but that the total number of fibres in this muscle is similar in the two breeds. Dumont & Schmitt (1970) obtain a mean fibre diameter for m. semimembranosus of the Pietrain of 88·7 μm, compared with 78·8 μm for the French Large White, and a mean fibre diameter of 78·8 μm for m. triceps brachii (lateral head) of the Pietrain compared with 73·6 μm for the Large White. The weight of m. semimembranosus, as a percentage of total muscle, is higher for the Pietrain, but the development of m. triceps brachii is similar in the two breeds. In these two studies, comparisons of individual muscle weights are not made at the same total muscle weight and comparisons of fibre diameters are not made at the same muscle weight or muscle TSA. As in the present study, the above results are based on measurements of fibres in unrestrained muscles. Possible differences in postmortem contraction between breeds must be eliminated by restraining contraction by splinting or the use of samples taken from muscles in rigor, before it can be concluded that the enhanced growth of m. longissimus (and presumably muscles of the femoral region such as m. semimembranosus) of the Pietrain is due to hypertrophy of a similar number of component fibres. Should this conclusion be validated, the muscularity of the Pietrain would differ from that of cattle with so-called 'muscular hypertrophy' which has been characterised as a hyperplasia by Ouhayoun & Beaumont (1968) in Charolais cattle and by MacKellar (1968) in South Devon cattle.

The results of Part 1 of this thesis indicate that the largest fibres of porcine muscle are fast-twitch, predominantly anaerobic fibres. It is reasonable to suppose that muscles with the highest mean fibre TSA contain

predominantly fibres of this type. In this connection, it is relevant that Schilling (1966) demonstrates a pattern of mean fibre diameter of muscles of the pig that corresponds to the pattern established for growth; the largest fibres are found in the femoral region, and muscles with smaller fibres are located cranially, and distally in the limbs. Although histochemical confirmation is needed, the hypothesis that high impetus muscles contain predominantly fast-twitch, anaerobic fibres appears reasonable. It would be of interest to determine if the muscularity of the Pietrain is due solely to a hypertrophy of this type of fibre.

It is difficult to resolve histochemically the question as to whether the selection of pigs for muscularity has resulted in some muscles becoming more highly dependent on anaerobic metabolism. Histochemical methods only determine the sites of activity of enzymes; the density of the sites so revealed gives a poor comparison of the overall enzymic activity of different samples. It is not unexpected, therefore, that reports comparing the muscle metabolism of normal and PSE-prone pigs by the proportions of histochemical fibre types are at variance. Although the area of a transverse section of m. longissimus occupied by aerobic fibres is shown to be greater in PSE-susceptible than in normal pigs by Sair, Lister, Moody, Cassens, Hoekstra & Briskey (1970), Cooper, Cassens & Briskey (1969) and Merkel (1971) find no difference in this parameter. Dildey, Aberle, Forrest & Judge (1970) show that the area of aerobic fibres is smaller in muscles becoming PSE postmortem. The discrepancy may be partly explained by the observation of Cooper, Cassens & Briskey (1969) and Sair, Kastenschmidt,

Cassens & Briskey (1972) that more of the aerobic fibres of PSE-prone pigs have an additional capacity for anaerobic metabolism, as shown by their high activity of GPase.

The present findings suggest that the variability within a breed may be too high to determine if breed differences in the proportion of myosin ATPase low fibres exist. Histochemical profiles are not established for the fibres of the Pietrain longissimus, but the above observations suggest that such a study cannot satisfactorily determine whether muscle anaerobiasis is a cause of the differences in postmortem glycolytic rates between breeds.

4.0 GENERAL DISCUSSION

This thesis shows that the techniques of muscle histochemistry and gross dissection of individual muscles can be combined to study the changes in muscle necessary for the support and propulsion of a progressively larger and sexually differentiating animal during growth. The use of these two methods therefore offers exciting possibilities for the elucidation of the developmental patterns of muscle in animals. This discussion briefly suggests some studies that might be attempted in the future to confirm the value of the techniques, to further our understanding of the changes involved during the growth of muscle, and to describe the growth of the muscles of meat animals in such a way that the development of breeds and species may be compared.

(1) The present study investigates the histochemical changes during the growth of m. longissimus and the diaphragm, in which the effects of increase in body size are not likely to be as evident as in a predominantly postural muscle. A study of the postnatal histochemical changes in a postural muscle of the pig (for example, m. extensor carpi radialis) might demonstrate more clearly the relationship between liveweight and the area of the muscle occupied by myosin ATPase low fibres, and the incidence and nature of 'transitional' fibres. The peculiar organisation of fibre types in porcine muscle might be used to advantage in a morphological study of growth changes in the relationship between nerve and muscle; cholinesterase histochemistry and the staining of nerve endings in serial sections would demonstrate the distribution of end plates in relation to the myosin ATPase low bundles,

and determine whether or not changes in the innervation of fibres are a normal consequence of growth.

(2) This thesis concludes that the histochemical changes observed in muscle during growth are mainly due to an adaption to the increased load applied. The pig, because of its great increase in body size during postnatal growth, is an ideal experimental animal with which to examine this hypothesis. However, the doubling of the work load of postural muscles achieved by amputation of a contralateral limb would produce a pronounced adaption in muscles of more convenient experimental animals (for example, m. soleus of the rat, cat or guinea-pig). Physiological studies should be made to confirm that the histochemical changes observed are accompanied by an increase in contraction time.

(3) Other aspects of the present histochemical study appear relevant to the understanding of muscle morphology. If the myofibrillar arrangements described in muscle fibres as 'Fibrillenstruktur' and 'Felderstruktur' by Krüger (1952) are, as suggested by this worker, correlated with 'phasic' and 'tonic' contraction respectively, this arrangement should also be related to the myosin ATPase activity of the fibres. If Krüger's method can be repeated consistently on frozen sections, this suggestion can be examined using serial sections.

The patterns of diformazan deposition observed in the present study differ between myosin ATPase high and myosin ATPase low fibres. It would be of interest to determine if mitochondrial and structural characteristics differ between fibres in which energy production proceeds at different rates,

by studying ultrastructure, mitochondrial enzyme histochemistry or biochemistry of muscles representing extremes of histochemical myosin ATPase activity. Such comparisons have usually been made on muscles differing only in their capacity for aerobic metabolism.

The dependence of the histochemical GPase reaction on the presence of tissue glycogen (paragraph 2.4.3) provides a method of determining the extent to which postmortem glycolysis has taken place in a particular fibre. By this means a link between the type of innervation and the metabolism of susceptible fibres could be investigated.

(4) The suggestion that a combined histochemical and dissection study can provide a functional explanation for the changes in muscle distribution occurring during the growth of meat producing animals can be tested by sampling a large number of muscles from a variety of situations throughout the carcass of the pig, and comparing the proportion of myosin ATPase high fibres of each muscle with its growth impetus. In particular, it would be of interest to investigate the existence of a craniocaudal gradient of increasing growth impetus and increasing density of myosin ATPase high fibres in the segmental units of m. multifidus.

(5) The existence of differences in muscle distribution between breeds suggested by the present study can be further examined by the investigation of extreme types of meat producing animals. For example, knowledge of the tissue distribution of the originally domestic pigs that have existed for many generations in the feral state in New Zealand would indicate what the effects of domestication, acting in reverse, have been in this species.

(6) The growth of individual muscles of female Pietrain and Large White pigs from birth to 64 kg liveweight can be attributed entirely to the effect of body size. The subsequent growth patterns of muscles of the female pig, and the differences effected by the hormone combinations of the other three sexes (entire male and gonadectomised male and female) have not been quantified. Growth gradients for cattle and sheep are not as clearly defined as for the pig (paragraph 3.4.2). A dissection study to demonstrate sex differences within these species, for example in Jersey cattle in which external differences in conformation appear particularly pronounced, will be necessary before patterns of growth in these animals can be fully understood. The sex differences in the muscle distribution of the guinea-pig (paragraph 3.4.4) should make this species suitable for a study of the possible differences between fibre types in the hormonal stimulation of muscle growth.

Despite significant advances in our ability to relate the molecular organisation of muscle to its function, we have little understanding of the factors influencing the development of this tissue. Thus the causes of the differences in muscle growth between breeds of meat producing animals remain as obscure as the outcome of a case of Duchenne muscular dystrophy is certain. In 1686, Newton found simple mathematical beauty in the motion of planetary systems. Biologists dare not expect such simplicity among organic systems, but they can hardly afford the luxury of superfluous causes.

REFERENCES

ALLEN, E., FORREST, J.C., CHAPMAN, A.B., FIRST, N., BRAG, R.W. & BRISKEY, E.J. (1966). Phenotypic and genetic associations between porcine muscle properties. Journal of Animal Science 25, 962-966.

ALTMAN, P.L. & DITTMER, D.S. (Eds.) (1962). Growth, including Reproduction and Morphological Development. Washington D.C.: Federation of American Societies for Experimental Biology.

ASHMORE, C.R. & DOERR, L. (1971). Comparative aspects of muscle fiber types in different species. Experimental Neurology 31, 408-418.

ASHMORE, C.R., TOMPKINS, G. & DOERR, L. (1972). Postnatal development of muscle fiber types in domestic animals. Journal of Animal Science 34, 37-41.

AUTRET, M. (1970). World protein supplies and needs. In Proteins as Human Food. Proceedings of the Sixteenth Easter School in Agricultural Science, University of Nottingham, 1969, pp. 3-19 (Ed. R.A. Lawrie). London: Butterworths.

BÁRÁNY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. Journal of General Physiology 50, 197-218.

BÁRÁNY, M. & CLOSE, R. (1971). The transformation of myosin in cross-innervated rat muscles. Journal of Physiology, London 213, 455-474.

BARNARD, R.J., EDGERTON, V.R., FURUKAWA, T. & PETER, J.B. (1971). Histochemical, biochemical and contractile properties of red, white and intermediate fibers. American Journal of Physiology 220, 410-414.

BARRETT, W. (1960). Moles and shrews. In Wild Animals of North America, pp. 321-325 (Eds. M.B. Grosvenor, M. Severy & R.M. McClung). Washington, D.C.: The National Geographical Society.

BEATTY, C.H., BASINGER, G.M. & BOCEK, R.M. (1967). Differentiation of red and white fibers in muscle from fetal, neonatal and infant rhesus monkeys. Journal of Histochemistry and Cytochemistry 15, 93-103.

BEECHER, G.R., CASSENS, R.G., HOEKSTRA, W.G. & BRISKEY, E.J. (1965). Red and white fibre content and associated postmortem properties of seven porcine muscles. Journal of Food Science 30, 969-976.

BEECHER, G.R., KASTENSCHMIDT, L.L., CASSENS, R.G., HOEKSTRA, W.G. & BRISKEY, E.J. (1968). A comparison of the light and dark portions of a striated muscle. Journal of Food Science 33, 84-88.

BENDALL, J.R. (1966). The effect of pre-treatment of pigs with curare on the postmortem rate of pH fall and the onset of rigor mortis in the musculature. Journal of the Science of Food and Agriculture 17, 333-338.

BENDALL, J.R. & LAWRIE, R.A. (1964). Watery pork. A discussion of symptoms and causes. Die Fleischwirtschaft 44, 416-421.

BENDALL, J.R. & VOYLE, C.A. (1967). A study of the histological changes in the growing muscles of beef animals. Journal of Food Technology 2, 259-283.

BERG, R.T. & BUTTERFIELD, R.M. (1966). Muscle:bone ratio and fat percentage as measures of beef carcass composition. Animal Production 8, 1-11.

BERG, R.T. & MUKHOTY, H.M. (1970). Lean distribution in carcasses from bulls, steers and heifers of various breeds. The 49th Annual Feeder's Day Report, 40-41. Department of Animal Science, University of Alberta, Edmonton.

BERTALANFFY, L. von & ESTWICK, R.R. (1953). Tissue respiration of musculature in relation to body size. American Journal of Physiology 173, 58-60.

BERTALANFFY, L. von & PIROZYNSKY, W.J. (1953). Tissue respiration, growth and basal metabolism. Biological Bulletin 105, 240-256.

BICHARD, M. (1968). Genetic aspects of growth and development in the pig. In Growth and Development of Mammals. Proceedings of the Fourteenth Easter School in Agricultural Science, University of Nottingham, 1967, pp. 309-326 (Eds. G.A. Lodge & G.E. Lamming). London: Butterworths.

BLANCHAER, M.C. (1964). Respiration of mitochondria of red and white skeletal muscle. American Journal of Physiology 206, 1015-1020.

BOCCARD, R. & DUMONT, B.L. (1970). Étude de l'accroissement relatif de la musculature en fonction de la vitesse de croissance corporelle chez l'agneau (Ovis aries). Compte rendu des séances de la Societé de biologie 164, 1251-1253.

BOCCARD, R., Le GUELTE, P. & ARNOUX, J. (1964). Influence de la vitesse de croissance sur la valeur des coefficients d'allométrie des tissus corporels de l'agneau. Compte rendu hebdomadaire des séances de l'Académie des sciences 258, 1908-1909.

BOCEK, R.M., BASINGER, G.M. & BEATTY, C.H. (1969). Glycogen synthetase, phosphorylase, and glycogen content of developing rhesus monkey. Pediatric Research 3, 525-531.

BOCEK, R.M. & BEATTY, C.H. (1966). Glycogen synthetase and phosphorylase in red and white muscle of rat and rhesus monkey. Journal of Histochemistry and Cytochemistry 14, 549-559.

BRISKEY, E.J. (1964). Etiological status and associated studies of pale, soft, exudative porcine musculature. Advances in Food Research 13, 89-178.

BRISKEY, E.J., FORREST, J.C. & JUDGE, M.D. (1966). Influence of ante-mortem factors on meat quality. Zeitschrift für Tierzüchtung und Züchtungsbiologie 82, 298-307.

BRISKEY, E.J., CASSENS, R.G. & MARSH, B.B. (Eds.) (1970). Physiology and Biochemistry of Muscle as a Food 2. Proceedings of an International Symposium sponsored by the University of Wisconsin, 1969. Madison: University of Wisconsin.

BRODY, S. (1927). Time relations of growth. III. Growth constants during the self-accelerating phase of growth. Journal of General Physiology 10, 637-664.

BRODY, S. (1945). Bioenergetics and Growth. Chapter 17. Linear growth, from and function, pp. 575-663. New York: Reinhold.

BROOKE, M.H. & ENGEL, W.K. (1966). Nitro blue tetrazolium: selective binding within striated muscle fibres. Neurology, Minneapolis 16, 799-806.

BROOKE, M.H. & KAISER, K.K. (1970). Muscle fiber types: How many and what kind ? Archives of Neurology 23, 369-379.

BULLER, A.J., ECCLES, J.C. & ECCLES, R.M. (1960a). Interactions between mononeurones and muscles in respect to the characteristic speeds of their responses. Journal of Physiology, London 150, 417-439.

BULLER, A.J., ECCLES, J.C. & ECCLES, R.M. (1960b). Differentiation of fast and slow muscles in the cat hind limb. Journal of Physiology, London 150, 399-416.

BULLER, A.J. & LEWIS, D.M. (1965). Further observations on the differentiation of skeletal muscles in the kitten hind limb. Journal of Physiology, London 176, 355-370.

BULLER, A.J., MOMMAERTS, W.F.H.M. & SERAYDARIAN, K. (1969). Enzymic properties of myosin in fast and slow twitch muscles of the cat following cross-innervation. Journal of Physiology, London 205, 581-597.

BULLER, A.J., MOMMAERTS, W.F.H.M. & SERAYDARIAN, K. (1971). Neural control of myofibrillar ATPase activity in rat skeletal muscle. Nature New Biology 233, 31-32.

BURKE, R.E., LEVINE, D.N., ZAJAC, F.E., TSAIRIS, P. & ENGEL, W.K. (1971). Mammalian motor units: physiological-histochemical correlation in three types in cat gastrocnemius. Science 174, 709-712.

BURTON, M. (1965). Systematic Dictionary of Mammals of the World. 2nd edition. London: Museum Press.

BUTTERFIELD, R.M. (1962). Prediction of muscle content of steer carcasses. Nature, London 195, 193-194.

BUTTERFIELD, R.M. (1964a). Estimation of carcass composition: the anatomical approach. In Carcase Composition and Appraisal of Meat Animals, Technical Conference, University of Melbourne, 1963, pp. 4-1 to 4-13 (Ed. D.E. Tribe). East Melbourne: CSIRO.

BUTTERFIELD, R.M. (1964b). Relative growth of the musculature of the ox. In Carcase Composition and Appraisal of Meat Animals, Technical Conference, University of Melbourne, 1963, pp. 7-1 to 7-20 (Ed. D.E. Tribe). East Melbourne: CSIRO.

BUTTERFIELD, R.M. (1966). Muscular hypertrophy of cattle. Australian Veterinary Journal 42, 37-39.

BUTTERFIELD, R.M. & BERG, R.T. (1966a). A classification of bovine muscles based on their relative growth patterns. Research in Veterinary Science 7, 326-332.

BUTTERFIELD, R.M. & BERG, R.T. (1966b). Relative growth patterns of commercially important muscle groups of cattle. Research in Veterinary Science 7, 389-393.

BUTTERFIELD, R.M. & BERG, R.T. (1966c). A nutritional effect on relative growth of muscles. Proceedings of the Australian Society of Animal Production 6, 298-304.

CAMERLYNCK, R. (1962). Le porc Piétrain à l'étranger. Revue de l'Agriculture, Bruxelles 15, 237-276.

CARDINET, G.H., FEDDE, M.R. & TUNELL, B.S. (1972). Correlates of histochemical and physiologic properties in normal and hypotrophic pectineus muscles of the dog. Laboratory Investigation 27, 32-38.

CARDINET, G.H., WALLACE, L.J., FEDDE, M.R., GUFFY, M.M. & BARDENS, J.W. (1969). Developmental myopathy in the canine with Type II muscle fiber hypotrophy. Archives of Neurology 21, 620-630.

CASSENS, R.G. & COOPER, C.C. (1971). Red and white muscle. Advances in Food Research 19, 1-74.

CASSENS, R.G., GIESLER, F.J. & KOLB, Q.E. (Eds.) (1972). The Proceedings of the Pork Quality Symposium. National Pork Quality Symposium, University of Wisconsin, 1972. Des Moines, Iowa: National Pork Producers Council.

CHARPENTIER, J. (1968). Glycogénolyse post mortem du muscle longissimus dorsi de porc. Annales de Zootechnie 17, 429-443.

CHARPENTIER, J., MONIN, G. & OLLIVIER, L. (1971). Correlations between carcass characteristics and meat quality in Large White pigs. In Proceedings of the 2nd International Symposium on Condition and Meat Quality of pigs, Zeist, 1971, pp. 255-260 (Eds. J.C.M. Hessel-deHeer, G.R. Schmidt, W. Sybesma & P.G. van der Wal). Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.

CHIAKULAS, J.J. & PAULY, J.E. (1965). A study of postnatal growth of skeletal muscle in the rat. Anatomical Record 152, 55-61.

CLOSE, R. (1964). Dynamic properties of fast and slow skeletal muscles of the rat during development. Journal of Physiology, London 173, 74-95.

CLOSE, R. (1969). Dynamic properties of fast and slow muscles of the rat after nerve cross-union. Journal of Physiology, London 204, 331-346.

COOPER, C.C., CASSENS, R.G. & BRISKEY, E.J. (1969). Capillary distribution and fiber characteristics in skeletal muscle of stress-susceptible animals. Journal of Food Science 34, 299-302.

COOPER, C.C., CASSENS, R.G., KASTENSCHMIDT, L.L. & BRISKEY, E.J. (1970). Histochemical characterisation of muscle differentiation. Developmental Biology 23, 169-184.

COOPER, C.C., CASSENS, R.G., KASTENSCHMIDT, L.L. & BRISKEY, E.J. (1971). Activity of some enzymes in developing muscle of the pig. Pediatric Research 5, 281-286.

CRABTREE, B. & NEWSHOLME, E.A. (1972). The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. Biochemical Journal 126, 49-58.

CUTHBERTSON, A. & POMEROY, R.W. (1962). Quantitative anatomical studies of the composition of the pig at 50, 68 and 92 kg carcass weight. II. Gross composition and skeletal composition. Journal of Agricultural Science 59, 215-223.

DAVIES, A.S. (1972a). Postnatal changes in the histochemical properties of porcine skeletal muscle. Journal of Anatomy 111, 487-489.

DAVIES, A.S. (1972b). Postnatal changes in the histochemical fibre types of porcine skeletal muscle. Journal of Anatomy (in press).

DAVIES, A.S. & GUNN, H.M. (1971). A comparative histochemical study of the mammalian diaphragm and m. semitendinosus. Journal of Anatomy 110, 137-139.

DAVIES, A.S. & GUNN, H.M. (1972). Histochemical fibre types in the mammalian diaphragm. Journal of Anatomy 112, 41-60.

DEHAYE, L. (1962). Dix années de sélection et d'observation de la race porcin Pietrain. Revue de l'Agriculture, Bruxelles 15, 35-52.

DIEM, K. & LENTNER, C. (Eds.) (1970). Documenta Geigy, Scientific Tables. Statistical methods, pp. 145-198. Seventh edition. Basle: J.R. Geigy.

DILDEY, D.D., ABERLE, E.D., FORREST, J.C. & JUDGE, M.D. (1970). Porcine muscularity and properties associated with pale, soft, exudative muscle. Journal of Animal Science 31, 681-685.

DORN, A. (1969). Studien zur Skeletmuskelentwicklung beim Meerschweinchen. II. Topochemiche Untersuchungen einiger Oxidoreduktasen. Acta Histochemica 33, 362-393.

DOW, J. & STRACHER, A. (1971). Changes in the properties of myosin associated with muscle development. Biochemistry, Easton 10, 1316-1321.

DUBOWITZ, V. (1965). Enzyme histochemistry of skeletal muscle. Journal of Neurology, Neurosurgery and Psychiatry 28, 516-524.

DUBOWITZ, V. (1967). Cross-innervated mammalian skeletal muscle: Histochemical physiological and biochemical observations. Journal of Physiology, London 193, 481-496.

DUBOWITZ, V. (1970). Differentiation of fiber types in skeletal muscle. In The Physiology and Biochemistry of Muscle as a Food 2, pp. 87-101 (Eds. E.J. Briskey, R.G. Cassens & B.B. Marsh). Madison: University of Wisconsin.

DUBOWITZ, V. & PEARSE, A.G.E. (1960a). Reciprocal relationship of phosphorylase and oxidative enzymes in skeletal muscle. Nature, London 185, 701-702.

DUBOWITZ, V. & PEARSE, A.G.E. (1960b). A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie 2, 105-117.

DUMONT, B.L. & BOCCARD, R. (1967). Critères modernes d'amelioration génétique des populations bovines dans le monde. Le rapport muscle/os, critère de sélection des bovins de boucherie. Atti della II Simposio Internationale di Zootechnia, Milano, 1967, pp. 149-155.

DUMONT, B.L., Le GUELTE, P. & ARNOUX, J. (1961a). Étude biometrique des bovins de boucherie. I. Variabilité de la composition anatomique de la carcasse des bovins Charolais. Annales de Zootechnie 10, 149-154.

DUMONT, B.L., Le GUELTE, P. & ARNOUX, J. (1961b). Étude biometrique des bovins de boucherie. II. Estimation du poids de la musculature chez les bovins Charolais. Annales de Zootechnie 10, 321-326.

DUMONT, B.L. & SCHMITT, O. (1970). Anatomie microscopique comparée du tissu musculaire squelettique de porcs Large-White et Piétrain. Annales de Génétique et de Sélection animale 2, 381-391.

DUMONT, B.L., SCHMITT, O. & ROY, G. (1969). Développement musculaire comparé de porcs Piétrain et Large White. Recueil de Médecine vétérinaire de l'École d'Alfort 145, 937-947.

DUNNILL, M.S. (1962). Postnatal growth of the lung. Thorax 17, 329-333.

EDGERTON, V.R. & SIMPSON, D.R. (1969). The intermediate muscle fiber of rats and guinea pigs. Journal of Histochemistry and Cytochemistry 17, 828-838.

EIKELENBOOM, G. (1972). Stress-susceptibility in swine and its relationship with energy metabolism in skeletal musculature. Thesis: Research Institute for Animal Husbandry 'Schoonoord', Zeist, Netherlands.

ELSLEY, F.W.H., McDONALD, I. & FOWLER, V.R. (1964). The effect of plane of nutrition on the carcasses of pigs and lambs when variations in fat content are excluded. Animal Production 6, 141-154.

ENESCO, M. & PUDDY, D. (1964). Increase in the number of nuclei and weight in skeletal muscles of rats of various ages. American Journal of Anatomy 114, 235-244.

ENGEL, W.K. (1962). The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. Neurology, Minneapolis 12, 778-794.

ENGEL, W.K. (1965). Diseases of the neuromuscular junction and muscle. In Neurohistochemistry, pp. 622-672 (Ed. C.W.M. Adams). Amsterdam: Elsevier.

ENGEL, W.K. (1970). Selective and nonselective susceptibility of muscle fiber types: a new approach to human neuromuscular diseases. Archives of Neurology 22, 97-117.

ENGEL, W.K., BROOKE, M.H. & NELSON, P.G. (1966). Histochemical studies of denervated or tenotomised cat muscle: illustrating difficulties in relating experimental animal conditions to human neuromuscular diseases. Annals of the New York Academy of Sciences 138, 160-185.

FAULKNER, J.A., MAXWELL, L.C., BROOK, D.A. & LIEBERMAN, D.A. (1971). Adaption of guinea pig plantaris muscle fibers to endurance training. American Journal of Physiology 221, 291-297.

FAULKNER, J.A., MAXWELL, L.C. & LIEBERMAN, D.A. (1972). Histochemical characteristics of muscle fibers from trained and detrained guinea pigs. American Journal of Physiology 222, 836-840.

FENICHEL, G.M. (1963). The B fiber of human fetal skeletal muscle. A study of fiber diameter size. Neurology, Minneapolis 13, 219-226.

FLOCK, D. (1968). Farbhellheit im m. long. dorsi als Selektionsmerkmal beim Schwein. Fleischwirtschaft 48, 1362-1365.

FORREST, J.C., WILL, J.A., SCHMIDT, G.R., JUDGE, M.D. & BRISKEY, E.J. (1968). Homeostasis in animals (Sus domesticus) during exposure to a warm environment. Journal of Applied Physiology 24, 33-39.

FOWLER, V.R. (1968). Body development and some problems of its evaluation. In Growth and Development of Mammals. Proceedings of the Fourteenth Easter School in Agricultural Science, University of Nottingham, 1967, pp. 195-211 (Eds. G.A. Lodge & G.E. Lamming). London: Butterworths.

FOWLER, V.R. & LIVINGSTONE, R.M. (1972). Modern concepts of growth in pigs. In Pig Production. University of Nottingham Eighteenth Easter School in Agricultural Science, 1971, pp. 143-161 (Ed. D.J.A. Cole). London: Butterworths.

GILLESPIE, C.A., SIMPSON, D.R. & EDGERTON, V.R. (1970). High glycogen content of red as opposed to white skeletal muscle fibers of guinea pigs. Journal of Histochemistry and Cytochemistry 18, 552-558.

GLASS, N.R. (1969). Discussion of calculation of power function with special reference to respiratory metabolism in fish. Journal of the Fisheries Research Board of Canada 26, 2643-2650.

GOEDBLOED, J.F. (1972). The embryonic and postnatal growth of rat and mouse. I. The embryonic and early postnatal growth of the whole embryo. A model with exponential growth and sudden changes in growth rate. Acta anatomica 82, 305-336.

GOLDSPINK, G. (1962). Studies on postembryonic growth and development of skeletal muscle. I. Evidence of 2 phases in which striated muscle fibres are able to exist. Proceedings of the Royal Irish Academy 62B, 135-150.

GOLDSPINK, G. (1969). Succinic dehydrogenase content of individual muscle fibers at different ages and stages of growth. Life Sciences 8(2), 791-808.

GOLLNICK, P.D. & KING, D.W. (1969). Effect of exercise and training on mitochondria of rat skeletal muscle. American Journal of Physiology 216, 1502-1509.

GUTH, L. & SAMAHA, F.J. (1969). Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. Experimental Neurology 25, 138-152.

GUTH, L. & SAMAHA, F.J. (1972). Erroneous interpretations which may arise from application of the 'myofibrillar ATPase' histochemical procedure to developing muscle. Experimental Neurology 34, 465-475.

GUTH, L., SAMAHA, F.J. & ALBERS, R.W. (1970). The neural regulation of some phenotypic differences between the fiber types of mammalian skeletal muscle. Experimental Neurology 26, 126-135.

GUTH, L. & YELLIN, H. (1971). The dynamic nature of the so-called 'fiber types' of mammalian skeletal muscle. Experimental Neurology 31, 277-300.

GUTMANN, E. & HÁJEK, I. (1971). Differential reaction of muscle to overload in compensatory hypertrophy and increased phasic activity. Physiologia Bohemoslovaca 20, 205-212.

GUTMANN, E., SCHIAFFINO, S. & HANZLIKOVÁ, V. (1971). Mechanism of compensatory hypertrophy in skeletal muscle of the rat. Experimental Neurology 31, 451-464.

HALL-CRAGGS, E.C.B. (1968). The contraction times and enzyme activity of two rabbit laryngeal muscles. Journal of Anatomy 102, 241-255.

HAMMOND, J. (1932). Growth and development of mutton qualities in the sheep. A survey of the problems involved in meat production. Edinburgh: Oliver and Boyd.

HARING, F., STEINBACH, J. & SCHEVEN, B. (1966). Untersuchungen über den mütterlichen Einfluss auf die prä- und post-natale Entwicklung von Schweinen extrem unterschiedlicher Grösse. I. Wachstumsstudien an reziproken Bastarden von Riese (Deutshes veredeltes Landschwein) und Zwerg (Vietnamese) im Vergleich zu Schweinen der Ausgangs populationen. Zeitschrift fur Tierzüchtung und Züchtungsbiologie 82, 37-53.

HESSEL-deHEER, J.C.M., SCHMIDT, G.R., SYBESMA, W. & van der WAL, P.G. (Eds.) (1971). Proceedings of the 2nd International Symposium on Condition and Meat Quality of Pigs, Zeist, 1971. Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.

HILL, A.V. (1950). The dimensions of animals and their muscular dynamics. Science Progress 38, 209-230.

HILL, A.V. (1965). Trails and Trials in Physiology. Chapter 6: The diffusion of oxygen through tissues, pp. 208-241. London: Edward Arnold.

HITZEMAN, J.W. (1963). Observations on the subcellular localisation of oxidative enzymes with nitro blue tetrazolium. Journal of Histochemistry and Cytochemistry 11, 62-70.

HOLLOSZY, J.O. (1967). Biochemical adaptation in muscle. Effects of exercise on mitocondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. Journal of biological Chemistry 242, 2278-2282.

HUXLEY, J.S. (1924). Constant differential growth-ratios and their significance. Nature, London 114, 895-896.

HUXLEY, J.S. (1932). Problems of Relative Growth. London: Methuen.

HWAI-PING, S. & HUGGINS, R.A. (1971). Growth of the beagle: changes in chemical composition. Growth 35, 369-376.

JACKSON, C.M. & LOWRY, L.G. (1912). On the relative growth of the component parts (head, trunk and extremities) and systems (skin, skeleton, musculature and viscera) of the albino rat. Anatomical Record 6, 449-474.

JAMES, N.T. (1971a). The distribution of muscle fibre types in fasciculi and their analysis. Journal of Anatomy 110, 335-342.

JAMES, N.T. (1971b). A geometrical probability study of type I muscle fibres in the rabbit and guinea pig. Journal of Neurological Sciences 14, 381-387.

JAMES, N.T. (1972). The histochemical properties of muscle fibres and the formation of subfasciculi in the tibialis anterior muscle of the rabbit. Journal of the Neurological Sciences 15, 429-437.

JASMIN, G., BOKDAWALA, F. & DESROSIERS, M. (1971). Identification de la fibre intermédiaire dans le muscle squelettique. L'Union médicale du Canada 100, 706-708.

JENNEKENS, F.G.I.,TOMLINSON, B.E. & WALTON, J.N. (1971a). The sizes of the two main histochemical fibre types in five limb muscles in man. An autopsy study. Journal of the Neurological Sciences 13, 281-292.

JENNEKENS, F.G.I., TOMLINSON, B.E. & WALTON, J.N. (1971b). Data on the distribution of fibre types in five human limb muscles. An autopsy study. Journal of the Neurological Sciences 14, 245-257.

JENSEN, P., CRAIG, H.B. & ROBISON, O.W. (1967). Phenotypic and genetic associations among carcass traits in swine. Journal of Animal Science 26, 1252-1260.

JINNAI, D. (1960). Functional differentiation of skeletal muscles. Acta medicinae Okayama 14, 159-169.

JUDGE, M.D., BRISKEY, E.J., CASSENS, R.G., FORREST, J.C. & MEYER, R.K. (1968). Adrenal and thyroid function in stress-susceptible pigs (Sus domesticus). American Journal of Physiology 214, 146-151.

JUDGE, M.D., CAHILL, V.R., KUNKLE, L.E. & BRUNER, W.H. (1959). Pork quality. I. Influences of some factors on pork muscle characteristics. Journal of Animal Science 18, 448-452.

JUDGE, M.D., FORREST, J.C., SINK, J.D. & BRISKEY, E.J. (1968). Endocrine related stress responses and muscle properties of swine. Journal of Animal Science 27, 1247-1253.

JUDGE, M.D. & MARPLE, D.N. (1971). Adrenal insufficiency in stress-susceptible pigs. In Proceedings of the 2nd International Symposium on Condition and Meat Quality of Pigs, Zeist, 1971, pp. 47-52 (Eds. J.C.M. Hessel-deHeer, G.R. Schmidt, W. Sybesma & P.G. van der Wal). Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.

JUNG, I. & BAULIEU, E.E. (1972). Testosterone cytosol 'receptor' in the rat levator ani muscle. Nature New Biology 237, 24-26.

KALLWEIT, E. (1969). Effects of environmental temperature and exercise ante-mortem on blood and meat quality characteristics in pigs. In Recent Points of View on the Condition and Meat Quality of Pigs for Slaughter. Proceedings of International Symposium on Meat Quality, Zeist, 1968, pp. 143-147 (Eds. W. Sybesma, P.G. van der Wal & P. Walstra). Zeist, Netherlands: Research Institute for Animal Husbandry.

KARPATI, G. & ENGEL, W.K. (1967a). Transformation of the histochemical profile of skeletal muscle by "foreign" innervation. Nature, London 215, 1509-1510.

KARPATI, G. & ENGEL, W.K. (1967b). Neuronal trophic function: a new aspect demonstrated histochemically in developing soleus muscle. Archives of Neurology 17, 542-545.

KAYER, C. & HEUSNER, A. (1964). Étude comparative du métabolisme énergétique dans la série animale. Journal de Physiologie, Paris 56, 489-524.

KENDRICK-JONES, J. & PERRY, S.V. (1967). The enzymes of adenine nucleotide metabolism in developing skeletal muscle. Biochemical Journal 103, 207-214.

KLEIBER, M. (1947). Body size and metabolic rate. Physiological Reviews 27, 511-541.

KOCHAKIAN, C.D., TILLOTSON, C. & AUSTIN, J. (1957). A comparison of the effect of inanition, castration and testosterone on the muscles of the male guinea pig. Endocrinology 60, 144-152.

KRAUS, H., KIRSTEN, R. & WOLFF, J.R. (1969). Die Wirkung von Schwimm- und Lauftraining auf die cellulăre Function und Struktur des Muskels. Archiv für die gesamte Physiologie des Menschen und der Tiere 308, 57-79.

KROES, Y. (1960). The Pietrain pig breed. Most important results of a practical investigation into the capability of this breed in Dutch surroundings. In La Race porcine belge Piétrain. Colloquium organised by the European Association for Animal Production, pp. 99-128. Bruxelles: Services des Informations agricoles.

KRÜGER, P. (1952). Tetanus und Tonus der quergestreiften Skelettmuskeln der Wirbeltiere und des Menschen. Leipzig: Akademische Verlagsgesellschaft.

KUGELBERG, E. & EDSTRÖM, L. (1968). Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres; relation to fatigue. Journal of Neurology, Neurosurgery and Psychiatry 31, 415-423.

LAIRD, A.K., TYLER, S.A. & BARTON, A.D. (1965). Dynamics of normal growth. Growth 29, 233-248.

LATZKOVITS, L. & DOMONKOS, J. (1965). The effect of postnatal development on the carbohydrate metabolism of tonic and tetanic muscles. Acta physiologica Academiae scientiarum hungaricae 28, 253-257.

LAWRIE, R.A. & GATHERUM, D.P. (1962). Studies on the muscle of meat animals. II. Differences in the ultimate pH and the pigmentation of longissimus dorsi muscles from two breeds of pigs. Journal of Agricultural Science 58, 97-102.

LAWRIE, R.A., GATHERUM, D.P. & HALE, H.P. (1958). Abnormally low ultimate pH in pig muscle. Nature, London 182, 807-808.

LEAN, I.J., CURRAN, M.K., DUCKWORTH, J.E. & HOLMES, W. (1972). Studies on Belgian Pietrain pigs. I. A comparison of Pietrain, Landrace and Pietrain Landrace crosses in growth, carcass characteristics and meat quality. Animal Production 15, 1-9.

LENDFERS, L.H.H.M. (1969). Transport and meat quality in pigs. In Recent Points of View on the Condition and Meat Quality of Pigs for Slaughter. Proceedings of International Symposium on Meat Quality, Zeist, 1968, pp. 193-199 (Eds. W. Sybesma, P.G. van der Wal & P. Walstra). Zeist, Netherlands: Research Institute for Animal Husbandry.

LESCH, M., PARMLEY, W.W., HAMOSH, M., KAUFMAN, S. & SONNENBLICK, E.H. (1968). Effects of acute hypertrophy on the contractile properties of skeletal muscle. American Journal of Physiology 214, 685-690.

LIEBERMAN, D.A., MAXWELL, L.C. & FAULKNER, J.A. (1972). Adaptation of guinea pig diaphragm to aging and endurance training. American Journal of Physiology 222, 556-560.

LISTER, D. (1971). Physiological aspects of meat quality and adaptation in pigs. European Association for Animal Production, Commission on Pig Production, Versailles, 1971.

LISTER, D. & RATCLIFF, P.W. (1971). The effects of pre-slaughter injection of magnesium sulphate on glycolysis and meat quality in the pig. In Proceedings of the 2nd International Symposium on Condition and Meat Quality of Pigs, Zeist, 1971, pp. 139-144 (Eds. J.C.M. Hessel-deHeer, G.R. Schmidt, W. Sybesma & P.G. van der Wal). Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.

LISTER, D., SAIR, R.A., WILL, J.A., SCHMIDT, G.R., CASSENS, R.G., HOEKSTRA, W.G. & BRISKEY, E.J. (1970). Metabolism of striated muscle of stress-susceptible pigs breathing oxygen or nitrogen. American Journal of Physiology 218, 102-107.

LISTER, D., SCOPES, R.K. & BENDALL, J.R. (1969). Some properties of the muscle of Pietrain pigs. Animal Production 11, 288.

LOHSE, C.L., MOSS, F.P. & BUTTERFIELD, R.M. (1971). Growth patterns of muscles of Merino sheep from birth to 517 days. Animal Production 13, 117-126.

LUDVIGSEN, J. (1957a). On the hormonal regulation of vasomotor reactions during exercise with special reference to the action of adrenal cortical steroids. Acta endocrinologica 26, 406-416.

LUDVIGSEN, J. (1957b). Akuter Herztod und Skelettmuskelentartung des Schweines. Archiv für expermentalle Veterinärmedizin 11, 198-224.

LUDVIGSEN, J. (1960). Maladaption syndromes in pigs. In Proceedings of the Second International Animal Nutritional Conference, Madrid, pp. 357-378.

LUITINGH, H.C. (1962). Developmental changes in beef steers as influenced by fattening, age and type of ration. Journal of Agricultural Science 58, 1-47.

MacCALLUM, J.B. (1898). On the histogenesis of the striated muscle fibre and the growth of the human sartorius muscle. Johns Hopkins Hospital Bulletin 9, 208-215.

MacDOUGALL, D.B. & DISNEY, J.G. (1967). Quality characteristics of pork with special reference to Pietrain, Pietrain x Landrace and Landrace pigs at different weights. Journal of Food Technology 2, 285-297.

MacKELLAR, J.C. (1968). Muscular hypertrophy in South Devon cattle. Thesis presented for diploma of Fellowship of the Royal College of Veterinary Surgeons.

MANN, W.S. & SALAFSKY, B. (1970). Enzymic and physiological studies on normal and disused developing fast and slow cat muscles. Journal of Physiology, London 208, 33-47.

MARKERT, C.J. & URSPRUNG, H. (1962). The ontogeny of isozyme patterns of lactate dehydrogenase in the mouse. Developmental Biology 5, 363-381.

MARRABLE, A.W..(1971). The Embryonic Pig: a Chronological Account. London: Pitman Medical.

McLOUGHLIN, J.V. & TARRANT, P.J.V. (1969). Post-mortem changes in muscle taken from live pigs immediately after slaughter. In Recent Points of View on the Condition and Meat Quality of Pigs for Slaughter. Proceedings of International Symposium on Meat Quality, Zeist, 1968, pp. 133-142 (Eds. W. Sybesma, P.G. van der Wal & P. Walstra). Zeist, Netherlands: Research Institute for Animal Husbandry.

McMEEKAN, C.P. (1940a). Growth and development in the pig, with special reference to carcass quality characters. I. Age changes in growth and development. Journal of Agricultural Science 30, 292-243.

McMEEKAN, C.P. (1940b). Growth and development in the pig, with special reference to carcass quality characters. II. The influence of the plane of nutrition on growth and development. Journal of Agricultural Science 30, 387-436.

McMEEKAN, C.P. (1940c). Growth and development in the pig, with special reference to carcass quality characters. III. Effect of the plane of nutrition on the form and composition of the bacon pig. Journal of Agricultural Science 30, 511-569.

McMEEKAN, C.P. (1943). Principles of Animal Production. Christchurch: Whitcombe and Tombs.

MEIJER, A.E.F.H. (1968a). Improved histochemical method for the demonstration of the activity of α-glucan phosphorylase. I. The use of glucosyl acceptor dextran. Histochemie 12, 244-252.

MEIJER, A.E.F.H. (1968b). Improved histochemical method for the demonstration of the activity of α-glucan phosphorylase. II. Relation of molecular weight of glucosyl acceptor dextran to activation of phosphorylase. Histochemie 16, 134-143.

MERKEL, R.A. (1971). The relationship of some cardiovascular and haematological parameters to porcine muscle quality. In Proceedings of the 2nd International Symposium on Condition and Meat Quality of Pigs, Zeist, 1971, pp. 97-103 (Eds. J.C.M. Hessel-deHeer, G.R. Schmidt, W. Sybesma & P.G. van der Wal). Wageningen, Netherlands: Centre for Agricultural Publishing and Documentation.

MILLER, I. & WEIL, W.B. (1963). Some problems in expressing and comparing body composition determined by direct analysis. Annals of the New York Academy of Sciences 110, 153-160.

MONTGOMERY, R.D. (1962). Growth of human striated muscle. Nature, London 195, 194.

MOODY, W.G. & CASSENS, R.G. (1968). Histochemical differentiation of red and white muscle fibers. Journal of Animal Science 27, 961-968.

MORITA, S., CASSENS, R.G. & BRISKEY, E.J. (1969). Localisation of myoglobin in striated muscle of the domestic pig: benzidine and $NADH_2$-TR reactions. Stain Technology 44, 283-286.

MORPURGO, B. (1898). Ueber die postembryonale Entwickelung der quergestreiften Muskeln von weissen Ratten. Anatomischer Anzeiger 15, 200-206.

MUKHOTY, H. & BERG, R.T. (1971). Influence of breed and sex on the allometric growth patterns of major bovine tissues. Animal Production 13, 219-227.

NACHLAS, M.M., TSOU, K., de SOUZA, E., CHENG, C. & SELIGMAN, A.M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. Journal of Histochemistry and Cytochemistry 5, 420-436.

NEWTON, I. (1686). Philosophiae naturalis Principia mathematica. Liber tertius: de mundi Systemate. London.

NISHIYAMA, A. (1966). Histochemical studies on the red, white and intermediate muscle fibers of some skeletal muscles. III. Histochemical demonstration of oxidative enzymes, phosphorylase and glycogen in respiratory muscle fibers. Acta medicinae Okayama 20, 137-146.

NOMINA ANATOMICA VETERINARIA (1968). Vienna: International Committee on Veterinary Anatomical Nomenclature.

NOVIKOFF, A.B., SHIN, W.Y. & DRUCKER, J. (1961). Mitochondrial localisation of oxidative enzymes. Staining results with two tetrazolium salts. Journal of biophysical and biochemical Cytology 9, 47-56.

NYSTRÖM, B. (1968). Histochemistry of developing cat muscles. Acta neurologica scandinavica 44, 405-439.

OGATA, T. (1958). A histochemical study of the red and white muscle fibers. Part I. Activity of the succinoxidase system in muscle fibers. Acta medicinae Okayama 12, 216-227.

OGATA, T. (1964). An electron microscopic study on the red, white and intermediate muscle fibers of the mouse. Acta medicinae Okayama 18, 271-280.

OLSON, C.B. & SWETT, C.P. (1969). Speed of contraction of skeletal muscle. The effect of hypoactivity and hyperactivity. Archives of Neurology 20, 263-270.

OMMER, P.A. (1971). Histochemical differentiation of skeletal muscle fibres in the bovine fetus. Experientia 27, 173-174.

OUHAYOUN, J. & BEAUMONT, A. (1968). Étude du caractère Culard. III. Anatomie microscopique comparée du tissue musculaire de mâles charolais normaux et culards. Annals de Zootechnie 17, 213-223.

PADYKULA, H.A. & GAUTHIER, G.F. (1963). Cytochemical studies of adenosine triphosphatases in skeletal muscle fibers. Journal of Cell Biology 18, 87-107.

PADYKULA, H.A. & HERMAN, E. (1955). The specificity of the histochemical method for adenosine triphosphatase. Journal of Histochemistry and Cytochemistry 3, 170-195.

PÁLSSON, H. (1955). Conformation and body composition. In Progress in the Physiology of Farm Animals 2, pp. 430-542 (Ed. J. Hammond). London: Butterworths.

PÁLSSON, H. & VERGÉS, J.B. (1952). Effects of the plane of nutrition on growth and the development of carcass quality in lambs. I. The effects of high and low planes of nutrition at different ages. II. Effects on lambs of 30 lb carcass weight. Journal of Agricultural Science 42, 1-149.

PAUL, M.H. & SPERLING, E. (1952). Cyclophorase system. XXIII. Correlation of cyclophorase activity and mitochondrial density in striated muscle. Proceedings of the Society for experimental Biology and Medicine 79, 352-354.

PIEPER, K.-S., FEUSTEL, G. & HÜBNER, H.-J. (1969). Zur Localisation der Succinodehydrogenase in "roten" (dünnen) Skelettmuskelfasern der weissen Ratte. Acta Histochemica 33, 171-178.

PREWITT, M.A. & SALAFSKY, B. (1970). Enzymic and histochemical changes in fast and slow muscles after cross-innervation. American Journal of Physiology 218, 69-74.

REEVE, E.C.R. (1940). Relative growth in the snout of anteaters. A study on the application of quantitative methods to systematics. Proceedings of the Zoological Society of London 110A, 47-80.

RICHMOND, R.J. & BERG, R.T. (1971a). Tissue development in swine as influenced by liveweight, breed, sex and ration. Canadian Journal of Animal Science 51, 31-39.

RICHMOND, R.J. & BERG, R.T. (1971b). Muscle growth and distribution in swine as influenced by liveweight, breed, sex and ration. Canadian Journal of Animal Science 51, 41-49.

ROBBINS, N., KARPATI, G. & ENGEL, W.K. (1969). Histochemical and contractile properties in the cross-innervated guinea pig soleus muscle. Archives of Neurology 20, 318-329.

ROMANUL, F.C.A. (1964). Enzymes in muscle: I. Histochemical studies of enzymes in individual muscle fibers. Archives of Neurology 11, 355-368.

ROMANUL, F.C.A. (1965). Capillary supply and metabolism of muscle fibers. Archives of Neurology 12, 497-509.

ROODYN, D.B. (1967). The mitochondrion. In Enzyme Cytology, pp. 103-180 (Ed. D.B. Roodyn). London: Academic Press.

RÜHL, B. (1971). Gewichte, Faserdicken und Kernzahlen des Herzmuskels und deren Beziehung zu Körpergewicht und Skelettmuskelmasse bei 205 Tage alten, 5 Rassen zugehörigen Schweinen. Zentralblatt für Veterinärmedizin 18A, 151-173.

SAIR, R.A., KASTENSCHMIDT, L.L., CASSENS, R.G. & BRISKEY, E.J. (1972). Metabolism and histochemistry of skeletal muscle from stress-susceptible pigs. Journal of Food Science 37, 659-663.

SAIR, R.A., LISTER, D., MOODY, W.G., CASSENS, R.G., HOEKSTRA, W.G. & BRISKEY, E.J. (1970). Action of curare and magnesium on striated muscle of stress-susceptible pigs. American Journal of Physiology 218, 108-114.

SAMAHA, F.J., GUTH, L. & ALBERS, R.W. (1970). The neural regulation of gene expression in the muscle cell. Experimental Neurology 27, 276-282.

SCARPELLI, D.G. & PEARSE, A.G.E. (1958). Cytochemical localisation of succinic dehydrogenase in mitochondria. Anatomical Record 132, 133-152.

SCHILLING, E. (1966). Muskelstruktur und Fleischqualität. Zeitschrift für Tierzüchtung und Züchtungsbiologie 82, 221-243.

SEEBECK, R.M. (1968). A dissection study of the distribution of tissues in lamb carcasses. Proceedings of the Australian Society of Animal Production 7, 297-302.

SEEBECK, R.M. & TULLOH, N.M. (1966). The representation of yield of dressed carcass. Animal Production 8, 281-288.

SELIGMAN, A.M., UENO, H., MORIZONO, Y., WASSERKRUG, H.L., KATZOFF, L. & HANKER, J. (1967). Electron microscopic demonstration of dehydrogenase activity with a new osmiophilic ditetrazolium salt (TC-NBT). Journal of Histochemistry and Cytochemistry 15, 1-13.

SKJERVOLD, H., STANDAL, N. & BRUFLOT, R. (1963). Effect of one form of exercise on the body development in pigs. Journal of Animal Science 22, 458-462.

STANT, E.G., MARTIN, T.G., JUDGE, M.D. & HARRINGTON, R.B. (1968). Physical separation and chemical analysis of the porcine carcass at 23, 46, 68 and 91 kilograms liveweight. Journal of Animal Science 27, 636-644.

STAUN, H. (1963). Various factors affecting number and size of muscle fibers in the pig. Acta agriculturae scandinavica 13, 293-322.

STEIN, J.M. & PADYKULA, H.A. (1962). Histochemical classification of individual skeletal muscle fibers of the rat. American Journal of Anatomy 110, 103-124.

STEINHAUF, D. (1969). Meat quality as a selection criterion. In Recent Points of View on the Condition and Meat Quality of Pigs for Slaughter, pp. 283-292 (Eds. W. Sybesma, P.G. van der Wal & P. Walstra). Zeist, Netherlands: Research Institute for Animal Husbandry.

SUCHENWIRTH, R. & BUNDSCHU, H.D. (1970). Enzymhistologische Befunde an der Skeletmuskulatur des Menschen. 1. Methoden und Ergebnisse bei Normalpersonen. Klinische Wochenschrift 48, 1096-1101.

SWANSON, M.A. (1948). Studies on the structure of polysaccharides. IV. Relation of the iodine colour to the structure. Journal of biological Chemistry 172, 825-837.

SYBESMA, W., van der WAL, P.G. & WALSTRA, P. (Eds.) (1969). Recent Points of View on the Condition and Meat Quality of Pigs for Slaughter. Proceedings of International Symposium on Meat Quality, Zeist, 1968. Zeist, Netherlands: Research Institutefor Animal Husbandry.

TAKEUCHI, T. (1956). Histochemical demonstration of phosphorylase. Journal of Histochemistry and Cytochemistry 4, 84.

TAKEUCHI, T. & KURIAKI, H. (1955). Histochemical detection of phosphorylase in animal tissues. Journal of Histochemistry and Cytochemistry 3, 153-160.

TAKEUCHI, T. & SASAKI, M. (1968). Histochemical and electron microscopic differences between native glycogen and polyglucose synthesized by phosphorylase in tissue cells. Acta Histochemica et Cytochemica 1, 63-78.

TENNEY, S.M. & REMMERS, J.E. (1963). Comparative quantitative morphology of the mammalian lung: diffusing area. Nature, London 197, 54-56.

THIELSCHER, H.H. (1966). Der Einfluss eines kontrollierten Lauftrainungs auf das Elektrokardiogramm bei Schweinen verschiedener Rassen. Zentralblatt für Veterinärmedizin 13A, 602-618.

THOMPSON, D'A.W. (1917). On Growth and Form 2, Second edition, 1942. Chapter 17: On the theory of transformations, or the comparison of related forms, pp. 1026-1095. Cambridge: University Press.

TODOROV, von, A. & PETROV, J. (1969). Entwicklung und Veränderung der Skelettmuskelfaser (Differenzierung, Hyperplasie und physiologische Degeneration) beim Schwein nach der Geburt. Anatomischer Anzeiger 125, 88-108.

TOPEL, D.G. (1969). Relation of plasma glucocorticoid levels to some physical and chemical properties of porcine muscle and the porcine stress syndrome. In Recent Points of View on the Condition and Meat Quality of Pigs for Slaughter. Proceedings of International Symposium on Meat Quality, Zeist, 1968, pp. 91-107 (Eds. W. Sybesma, P.G. van der Wal & P. Walstra). Zeist, Netherlands: Research Institute for Animal Husbandry.

TOPEL, D.G., MERKEL, R.A. & WISMER-PEDERSEN, J. (1967). Relationship of plasma 17-hydroxycorticosteroid levels to some physical and biochemical properties of porcine muscle. Journal of Animal Science 26, 311-315.

TRAYER, I.P. & PERRY, S.V. (1966). The myosin of developing skeletal muscle. Biochemische Zeitschrift 345, 87-100.

TULLOH, N.M. (1964). The carcass compositions of sheep, cattle and pigs as functions of body weight. In Carcase Composition and Appraisal of Meat Animals. Technical Conference, University of Melbourne, 1963, pp. 5-1 to 5-30 (Ed. D.E. Tribe). East Melbourne: CSIRO.

UNSHELM, J., KALLWEIT, E., OLDIGS, B., SCHRÖDER, J., PFEIDERER, U.-E. & SCHUTZBAR, W.v. (1972). Untersuchungen über die Mastleistung und Ausschlachtungsbefunde bei Schweinen unterschiedlicher Nutzungsrichtung und Grösse. Züchtungskunde 44, 42-55.

VACCARO, R. & DILLARD, E.U. (1966). Relationship of dam's weight and weight changes of calf's growth rate in Hereford cattle. Journal of Animal Science 25, 1063-1068.

Van Den HENDE, C., MUYLLE, E., OYAERT, W. & de ROOSE, P. (1971). The respiration of heart muscle and skeletal muscle in vitro. Zentralblatt für Veterinärmedizin 18A, 709-716.

Van Den HENDE, C., MUYLLE, E., OYAERT, W. & de ROOSE, P. (1972) Changes in muscle characteristics in growing pigs. Histochemical and electron microscopic study. Zentralblatt für Veterinärmedizin 19A, 102-110.

Van SNICK, J. & CAMERLYNCK, R. (1966). Le porc Piétrain. World Review of Animal Production 1966, No. 3, 95-111.

VOLD, E., STEINHAUF, D. & WENIGER, J.H. (1965). Postmortale Veränderungen der Fleischbeschaffenheit beim Schwein, zugleich ein weiterer Beitrag zur Methodik der Fleischqualitätsuntersuchung. Fleischwirtschaft 45, 938-943.

VRBOVÁ, G. (1963). Changes in the motor reflexes produced by tenotomy. Journal of Physiology, London 166, 241-250.

WAINMAN, P. & SHIPOUNOFF, G.C. (1941). The effects of castration and testosterone proprionate on the striated perineal musculature of the rat. Endocrinology 29, 975-978.

WALKER, D.E. (1961). A study of the growth and development of Jersey cattle. I. A new carcass dissection technique. New Zealand Journal of Agricultural Research 4, 99-122.

WALLACE, L.R. (1948). The growth of lambs before and after birth in relation to the level of nutrition. Journal of Agricultural Science 38, 93-153, 243-302, 367-401.

WALTON, A. & HAMMOND, J. (1938). Maternal effects on growth and conformation in Shire horse and Shetland pony crosses. Proceedings of the Royal Society 125B, 311-335.

WELCKER, H. & BRANDT, A. (1903). Gewichtswerte der Körperorgane bei dem Menschen und den Tieren. Ein Beitrag zur vergleichenden Anatomie und Entwicklungsgeschichte. Archiv für Anthropologie 28, 1-89.

WILLEMS, A.E.R. (1960). Origine et évolution du porc belge de Piétrain. In La Race porcine belge Piétrain. Colloque organisé par la Federation Européenne de Zootechnie, pp. 5-9. Bruxelles: Service des Informations agricoles.

WIRSEN, C. & LARSSON, K.S. (1964). Histochemical differentiation of skeletal muscle in fetal and newborn mice. Journal of Embryology and Experimental Morphology 12, 759-767.

WISMER-PEDERSEN, J. (1968). Modern production practices and their influence on stress conditions. In The Pork Industry: Problems and Progress, pp. 163-176 (Ed. D.G. Topel). Ames: Iowa State University Press.

WOHLFART, G. (1937). Über das Vorkommen verschiedener Arten von Muskelfasern in der Skelettmuskulatur des Menschen und einiger Säugetiere. Acta psychiatrica et neurologica, Supplementum 12, 1-119.

APPENDIX 1

Worksheet for recording data obtained from the total muscle dissection of the pig. All weights are recorded in grammes.

A.	Breed and sex	L.W. Female
	Identification	8250
	Date of birth	16 Jun '71
	Date of slaughter	11 Aug '71
	Age at slaughter (days)	56
	Liveweight at slaughter	12950
	Carcass weight	9580
B.	Lungs	165
	Heart	62·0
	Liver	367
	Spleen	20·0
	Stomach	125
	Small intestine	650
	Large intestine	270
	Gut content	627
	Uterine horns and ovaries	2·50
	Half eviscerated carcass weight (right-hand-side, including kidney and half head)	4750
C.	Kidney	27·2
	Adrenal gland	0·460
	Abdominal fat and loose connective tissue	25·0
	(1) Diaphragm	17·4
	(2) Transversus abdominis	35·3
	(3) Rectus abdominis	39·0

Half carcass divided by cutting between T15 and L1, through M. longissimus, m. obliquus externus abdominis and m. iliocostalis. M. psoas major is reflected.

Weight of hindquarter	1790
Weight of forequarter and half head	2816
TOTAL HALF CARCASS WEIGHT	4750
ORIGINAL HALF CARCASS WEIGHT (B)	4750
% LOSS	-

D.	(4) Cutaneus trunci	21·0
	(5) Obliquus externus abdominis (part)	23·5
	(6) Obliquus internus abdominis	18·9
	(7) Tensor fasciae latae	19·1
	(8) Biceps femoris	149·0
	(9) Semitendinosus	38·9
	(10) Gluteus superficialis	35·6
	(11) Gluteus medius	31·3
	(12) Gluteus accessorius	20·0
	(13) Gracilis	23·1
	(14) Sartorius	2·81
	(15) Semimembranosus	114
	(16) Pectineus	9·18
	(17) Adductor	39·1
	(18) Quadratus femoris	1·91
	(19) Psoas major) Iliacus)	48·4
	(20) Psoas minor	5·37
	(21) Obturator externus and gemelli	13·2
	(22) Gluteus profundus	9·65
	(23) Iliocostalis lumborum	0·446
	(24) Longissimus lumborum	78·3
	(25) Interspinalis lumborum) Intertransversarius lumborum) Multifidus lumborum)	31·5
	(26) Rectus femoris	49·0
	(27) Vastus lateralis) Vastus intermedius) Vastus medialis)	82·2
	(28) Soleus) Gastrocnemius)	54·8
	(29) Flexor digitorum superficialis	9·27
	(30) Popliteus	6·85
	(31) Flexor digitorum profundus	21·1
	(32) Fibularis longus	4·52
	(33) Fibularis tertius) Extensor digitorum longus (3 heads))	8·91
	(34) Tibialis cranialis	4·36
	(35) Extensor digiti I longus	0·452
	(36) Extensor digitorum lateralis	4·69
	(37) Extensor digitorum brevis	2·63
	(38) Interossei and Adductors of III and IV	2·29
	TOTAL MUSCLE WEIGHT	985·338

E. Lumbar and sacral vertebrae (undivided)) 122
Half pelvis)
Femur 72·8
Patella 3·75
Tibia and Fibula 51·9
Tarsus and metatarsus 63·7
TOTAL BONE WEIGHT 314·15

	Length (cm)	Diameter (cm)
Femur	11·9	1·3
Tibia	10·5	1·4
Metatarsus IV	5·6	1·5
Lumbar vertebrae	12·5	

F. Intermuscular fat and fascia 49·0
Subcutaneous fat 140
TOTAL FAT WEIGHT 189

G. Integument (skin, digits, tail) 243
Tendons, fascia, ligaments 36·0
Scrap (blood vessels, nerves, lymph nodes) 7·5
TOTAL 286·5

H. Total weight of muscles (D) 985
Total weight of bones (E) 314
Total weight of fat (F) 189
Total miscellaneous (G) 286
TOTAL WEIGHT HINDQUARTER 1774
ORIGINAL WEIGHT HINDQUARTER (C) 1790
% LOSS 0·8%

I. Back fat thickness at thoracolumbar junction (cm) 0·3
Longissimus at thoracolumbar junction:
width (cm) 4·9
depth (cm) 2·3

J.

(1)	Cutaneus omobrachialis) Cutaneus colli)	38·5
(2)	Trapezius	21·3
(3)	Brachiocephalicus	24·2
(4)	Omotransversarius	3·40
(5)	Rhomboideus capitis) cervicis) thoracis)	23·8
(6)	Pectoralis superficialis	16·7
(7)	Pectoralis ascendens	67·1
(8)	Pectoralis cleidoscapularis	35·5
(9)	Latissimus dorsi	47·5
(10)	Teres major	12·1
(11)	Supraspinatus	51·3
(12)	Infraspinatus	39·5
(13)	Deltoideus	7·69
(14)	Tensor fasciae antebrachii	6·66
(15)	Subscapularis	16·8
(16)	Coracobrachialis	2·66
(17)	Biceps brachii	9·41
(18)	Articularis humeri	1·66
(19)	Teres minor	4·11
(20)	Triceps brachii - long head	71·2
(21)	- medial head	9·70
(22)	- lateral head	20·4
(23)	Brachialis	12·9
(24)	Extensor carpi radialis	14·4
(25)	Anconeus	3·80
(26)	Flexor carpi ulnaris	1·39
(27)	Flexor carpi radialis	2·84
(28)	Pronator teres	·003
(29)	Supinator	·392
(30)	Flexor digitorum profundus (3 heads)) " " superficialis (2 heads))	26·1
(31)	Extensor digitorum communis (3 heads)	4·67
(32)	" " lateralis (2 heads)	3·90
(33)	Ulnaris lateralis	1·69
(34)	Extensor carpi obliquus	1·11
(35)	Abductors, adductors and flexors of II & V) Interosseus of III & V)	2·13

K.	(36) Obliquus externus abdominis (part)		31·3
	(37) Serratus ventralis (cranialis and candalis)		78·7
	(38) Scalenus dorsalis and ventralis		11·2
	(39) Serratus dorsalis (cranialis and caudalis)		5·82
	(40) Iliocostalis thoracis		10·8
	(41) Longissimus thoracis and cervicis		122
	(42) Splenius occipitalis)		
	temporalis)		19·8
	cervicalis)		
	(43) Semispinalis capitis - Biventer cervicis		22·5
	(44) - Complexus		21·4
	(45) Spinalis		21·2
	(46) Sternomastoideus		6·55
	(47) Sternohyoideus)		7·54
	Sternothyroideus)		
	(48) Rectus capitis (dorsalis and ventralis))		
	Obliquus capitis (caudalis and cranialis))		
	Longus colli)		
	Intertransversarii cervicis)		58·9
	Multifidus thoracis and cervicis)		
	Interspinales)		
	Rotatores)		
	Levatores costarum)		
	(49) Transversus thoracis)		
	Rectus thoracis)		79·2
	Intercostales)		
	TOTAL MUSCLE WEIGHT (J AND K)		1103·425

L.	Cervical and thoracic vertebrae)		234
	Ribs and sternum)		
	Scapula		34·5
	Humerus		64·3
	Radius and Ulna		48·6
	Carpus and Metacarpus		35·4
	TOTAL BONE WEIGHT		416·8

	Length (cm)	Width (cm)
Scapula	10·6	1·6
Humerus	11·4	1·5
Radius	12·1	2·2
Metacarpus IV	4·6	1·5
Thoracic vertebrae	26·3	
Rib I	4·9	
Rib VIII	11·2	

M.	Intermuscular fat and fascia	76·0
	Subcutaneous fat	277
	TOTAL FAT WEIGHT	353
N.	Integument (skin and digits)	214
	Tendons, fascia and ligaments	14·0
	Scrap (blood vessels, nerves, lymph nodes)	39
	Half head	598
	TOTAL	865
O.	Total weight of muscles (J and K)	1103
	Total weight of bones (L)	417
	Total weight of fat (M)	353
	Total miscellaneous (N)	865
	TOTAL WEIGHT FOREQUARTER	2738
	ORIGINAL WEIGHT FOREQUARTER (C)	2816
	% LOSS	2·8
P.	(50) Levator labii maxillaris	3·30
	(51) Lateralis nasi	1·27
	(52) Depressor labii maxillaris	2·18
	(53) Digastricus	2·21
	(54) Masseter	19·3
	(55) Temporalis	10·7
	(56) Pterygoideus (lateralis and medialis)	8·37
	TOTAL MUSCLES OF MASTICATION (53-56)	47·33
	Muscles and bone of head are not included in totals of muscle and bone weight	
Q.	GRAND TOTAL muscle (C, D, J and K)	2180
	GRAND TOTAL bone (E and L)	731
	GRAND TOTAL fat (C, F and M)	567
	GRAND TOTAL miscellaneous (G and N)	1151
	Kidney and adrenal gland	27·7
	TOTAL WEIGHT OF HALF CARCASS	4658
	ORIGINAL WEIGHT	4750
	% LOSS	1·9

APPENDIX 2. DISSECTION DATA: PIETRAIN

	1 (Q)	2 (O)	3 (L)	4 (E)	5 (N)	6 (I)	7 (F)	8 (P)	9 (K)	10 (S)	11 (C)	12 (R)	13 (H)	14 (J)	15 (M)	16 (G)	17 (D)	18 (U)
LIVEWEIGHT (kg.)	1·670	1·721	2·106	2·500	3·562	4·000	7·500	8·265	8·766	13·814	17·700	17·727	34·000	34·500	37·300	70·000	72·300	72·700
AGE AT SLAUGHTER (days)	9	13	9	16	16	21	34	28	43	61	74	67	100	117	102	164	157	145
DATE OF BIRTH.	15·7·70	4·7·70	1·7·70	10·6·70	1·7·70	12·6·70	23·5·70	26·6·70	28·5·70	29·5·70	5·4·70	23·5·70	26·3·70	24·3·70	5·4·70	19·1·70	19·1·70	24·1·70
COLD CARCASS WEIGHT (kg.)	1·753	1·387	1·756	2·179	2·923	3·378	6·410	7·130	7·550	11·628	13·100	14·576	25·800	26·500	27·800	59·000	56·300	57·500
HALF CARCASS WEIGHT (kg.)	0·651	0·680	0·830	1·050	1·465	1·614	3·390	3·522	3·714	5·650	7·160	7·207	12·500	13·100	13·657	29·000	27·000	26·500
WEIGHT OF VISCERA.																		
HEART. (g).	12·1	11·4	11·2	18·6	19·5	24·5	42·8	42·1	49·8	65·5	92·0	175	128	153	134	*	213	291
LIVER. (g.)	47·6	50·3	65·3	66·8	108	104	183	169	207	379	404	415	287	868	926	*	1,507	1,283
SPLEEN. (g).	2·12	3·54	3·97	3·5	6·26	6·77	19·6	14·3	17·2	30·6	37·0	53·3	63·0	52·0	37·6	*	108	94·0
STOMACH. (g.)	14·2	12·3	16·7	17·2	24·0	22·1	46·8	37·5	55·1	96·7	152	162	221	250	267	*	417	359
SMALL INTESTINE. (g.)	81·3	74·3	468	122	120	191	317	294	264	484	600	620	1,005	425	990	*	1,475	1,186
LARGE INTESTINE. (g.).	54·5	36·3	36·8		64·5	54·0	75·2	145	119	251	330	368	632	333	873	*	1,076	1,378
GUT CONTENT. (g.)	149	180	358	26·9	64·0	37·6	181	111	122	363	250	542	1,354	2,995	1,525	*	2,375	1,902
UTERINE HORNS AND OVARIES (g.)	0·992	1·70	1·07	0·8	3·14	1·42	2·4	2·49	3·34	6·36	13·0	11·2	40·2	32·0	92·5	*	141	86
KIDNEY.	5·78	6·26	6·61	5·83	12·1	11·4	18·7	19·6	17·5	33·0	35·7	41·7	71·1	72·5	76·5	*	118	126
ADRENAL.	0·071	0·155	0·070	0·074	0·359	0·500	0·273	0·326	0·416	0·664	0·664	0·671	0·710	0·896	0·888	*	1·27	1·11
HINDQUARTER WEIGHT. (kg.)	0·197	*	0·278	0·367	0·494	0·573	1·200	1·323	1·463	2·270	3·100	2·800	4·920	5·140	5·488	11·730	11·580	11·040
FOREQUARTER WEIGHT. (kg.)	0·434	*	0·520	0·644	0·913	0·898	1·716	2·067	2·148	3·150	4·060	4·105	6·620	7·110	7·317	15·900	14·740	14·440
AXIAL SKELETON MUSCLE WEIGHTS.																		
(a) HEAD.																		
M. LEVATOR LABII MAXILLARIS.	0·917	0·705	0·959	0·932	1·36	1·39	2·55	2·72	3·57	4·46	5·98	4·35	8·24	9·55	8·68	23·8	18·0	17·8
M. LATERALIS NASI.	0·542	0·432	1·31	0·985	0·496	1·22	1·17	1·16	1·85	1·63	2·45	2·63	6·48	5·19	3·40	7·78	6·43	8·35
M. DEPRESSOR LABII MAXILLARIS.	0·575	0·525	0·617	0·625	1·02	1·10	1·87	2·22	2·76	3·01	2·83	3·08	6·42	5·65	6·43	9·13	8·97	10·8
M. DIGASTRICUS.	0·572	0·478	0·722	0·718	1·07	1·28	1·64	1·91	3·21	2·52	2·25	3·56	5·61	5·39	5·47	13·7	12·2	13·3
M. MASSETER.	2·70	2·97	4·21	3·76	5·12	7·31	10·3	13·5	16·5	24·6	32·0	28·7	43·3	47·1	54·7	98·0	85·4	124
M. TEMPORALIS.	1·87	1·68	2·17	3·08	3·82	3·65	6·92	4·63	10·3	10·9	16·0	13·7	25·3	26·8	18·7	59·4	62·0	66·6
M. PTERYGOIDEUS.	1·28	1·11	1·69	1·18	2·82	2·64	5·83	4·22	7·89	7·65	12·8	10·4	16·3	15·0	15·9	34·1	40·9	57·4
(b) NECK AND THORAX.																		
M. SCALENUS.	0·669	1·18	2·37	1·97	1·54	3·54	10·6	5·90	11·4	10·2	20·2	18·0	*	24·9	13·3	56·7	70·5	61·9
M. ILIOCOSTALIS THORACIS.	1·85	1·81	2·33	2·37	3·73	3·37	8·46	10·2	12·5	16·7	22·6	21·1	37·2	31·1	30·9	91·1	84·8	82·9
M. LONGISSIMUS THORACIS.	11·3	12·5	20·0	24·3	36·5	31·7	98·1	79·4	117	189	219	234	388	370	363	1,057	1,055	934
M. SPLENIUS CERVICIS.	4·79	6·10	5·51	6·05	7·51	9·16	25·9	24·4	20·8	39·9	44·7	46·1	79·2	62·3	51·0	179	141	166
M. SEMISPINALIS-BIVENTER CERVICIS.	5·28	3·17	4·71	5·34	7·19	5·20	20·1	16·4	18·8	29·5	43·8	27·8	53·5	69·9	66·9	192	128	129
" " - COMPLEXUS.	3·87	3·63	4·63	5·29	7·42	6·59	19·6	13·4	22·6	35·6	30·7	25·6	49·0	41·2	57·3	121	128	114
M. SPINALIS.	3·90	3·63	4·72	5·81	9·26	6·99	21·2	24·6	26·6	18·0	60·0	48·4	68·7	76·2	71·0	254	207	176
M. STERNOMASTOIDEUS.	0·601	1·52	2·14	2·13	2·90	1·81	2·64	5·85	7·18	*	8·89	14·1	19·2	18·0	19·6	*	*	47·4

DISSECTION DATA: PIETRAIN

	1 (Q)	2 (O)	3 (L)	4 (E)	5 (N)	6 (I)	7 (F)	8 (P)	9 (K)	10 (S)	11 (C)	12 (R)	13 (H)	14 (J)	15 (M)	16 (G)	17 (D)	18 (B)
M. STERNO-HYOIDEUS + STERNO-THYROIDEUS.	1·74	1·36	2·67	1·79	3·28	*	*	6·11	4·06	*	27·5	18·9	*	18·9	24·9	*	*	58·2
DEEP NECK MUSCLES.	9·25	7·58	*	11·5	19·9	13·2	38·1	46·2	40·6	53·1	95·0	89·0	167	212	217	536	354	318
Mm. INTERCOSTALES EXTERNI et INTERNI.	10·4	10·0	11·8	19·2	26·5	21·2	42·1	54·2	70·7	100	112	158	253	274	315	650	536	575
(c) LUMBO-SACRAL REGION.																		
M. PSOAS MINOR.	0·91	1·18	0·475	6·50	1·39	2·88	2·23	3·29	3·36	6·67	7·45	9·15	21·2	14·3	12·8	29·3	24·0	34·3
M. ILIOCOSTALIS LUMBORUM.	*	*	*	*	0·294	*	*	0·468	3·08	0·863	3·80	0·818	*	*	3·39	1·45	3·82	3·86
M. LONGISSIMUS LUMBORUM.	5·78	7·27	9·06	17·0	15·9	25·4	50·0	59·6	60·7	126	171	144	267	264	145	743	611	676
DEEP LUMBAR MUSCLES.	1·61	2·17	1·84	4·13	5·78	4·93	20·3	8·61	24·2	24·7	13·1	18·6	54·8	46·7	106	235	225	181
(d) ABDOMINAL MUSCLES.																		
DIAPHRAGMA.	4·44	3·63	5·17	5·05	10·1	7·31	18·5	18·3	19·5	37·1	41·2	54·6	69·5	70·1	59·9	62·7	58·2	95·8
M. TRANSVERSUS ABDOMINIS.	3·16	4·24	5·65	5·08	12·4	10·2	24·3	23·9	20·8	40·5	65·5	62·8	101	122	108	210	257	285
M. RECTUS ABDOMINIS.	4·59	4·02	6·88	6·08	11·1	14·5	29·1	32·2	30·2	61·7	67·8	77·1	106	133	119	258	277	293
M. OBLIQUUS EXTERNUS ABDOMINIS.	4·78	6·45	10·8	8·86	14·5	16·6	37·5	29·7	34·0	57·4	83·6	99·0	146	122	146	379	340	377
M. OBLIQUUS INTERNUS ABDOMINIS.	3·33	2·61	4·21	4·67	7·36	7·58	18·1	16·0	18·9	27·9	42·0	41·5	80·0	75·8	79·1	164	177	205
(e) CUTANEOUS MUSCLES.																		
M. CUTANEUS TRUNCI.	1·57	3·32	1·55	1·98	5·32	6·40	8·20	17·1	17·5	31·8	34·7	32·0	58·6	61·6	74·2	210	157	177
M. CUTANEUS OMOBRACHIALIS + COLLI.	5·44	1·92	9·04	3·68	13·5	8·47	23·3	32·9	32·3	74·5	57·8	62·5	86·5	100	122	277	348	241
APPENDICULAR SKELETON MUSCLE WEIGHTS																		
M. TRAPEZIUS. (a FORELIMB)	4·06	3·62	5·45	3·89	9·73	10·5	21·0	24·5	31·0	33·7	42·6	43·4	67·0	67·4	67·7	175	157	164
M. BRACHIOCEPHALICUS.	3·92	3·94	3·30	5·51	9·26	9·20	19·8	19·5	20·0	32·4	39·6	44·5	68·1	72·2	66·9	147	156	146
M. OMOTRANSVERSARIUS.	0·210	1·12	0·395	0·643	0·826	0·229	1·77	2·36	2·74	3·56	3·87	1·91	6·50	5·78	7·81	17·1	16·4	15·2
M. RHOMBOIDEUS.	5·69	2·53	5·41	5·51	7·63	8·54	17·0	17·0	18·3	21·9	20·7	38·4	49·2	65·4	57·3	109	77·7	102
M. PECTORALIS SUPERFICIALIS.	2·20	2·65	2·72	3·43	5·22	4·46	6·21	12·5	15·2	18·6	33·3	29·8	33·2	43·3	49·9	132	89·2	106
M. PECTORALIS ASCENDENS.	7·46	10·2	11·8	12·7	26·0	22·4	37·1	21·4	53·8	72·8	96·1	93·5	159	164	210	393	365	440
M. PECTORALIS CLEIDOSCAPULARIS.	3·98	2·41	5·95	7·31	7·61	9·94	22·8	56·0	24·5	38·5	47·1	33·5	65·2	87·8	49·5	156	194	145
M. LATISSIMUS DORSI.	6·58	7·38	10·0	13·2	18·0	17·2	34·5	36·8	43·7	66·4	71·2	75·8	116	119	117	354	321	285
M. SERRATUS VENTRALIS.	11·2	12·0	10·2	16·7	30·2	29·2	59·8	633	71·2	109	156	151	261	216	257	560	532	681
M. SERRATUS DORSALIS.	1·19	0·957	3·32	1·84	4·18	4·54	9·66	6·03	9·06	14·1	17·1	11·8	24·4	22·9	19·5	66·2	48·2	25·2
M. TERES MAJOR	1·40	2·69	2·69	3·08	4·65	5·39	10·6	11·4	12·6	19·9	20·9	24·9	39·5	38·8	42·4	92·4	81·9	133
M. TERES MINOR.	0·921	0·828	0·850	0·811	1·29	1·35	2·88	3·54	3·91	6·26	14·1	8·74	15·7	15·1	14·1	25·3	24·4	25·8
M. SUPRASPINATUS	9·38	9·02	11·6	12·8	20·8	17·8	34·1	43·5	45·6	44·1	73·9	80·0	151	146	126	302	322	290
M. INFRASPINATUS	6·53	6·09	6·99	8·40	14·4	18·9	26·3	31·7	37·8	65·1	66·4	74·1	117	130	141	260	314	240
M. DELTOIDEUS.	1·14	1·50	1·91	1·93	2·89	3·64	7·70	7·11	9·14	10·7	7·55	17·7	25·0	20·2	24·6	63·8	62·5	51·8
M. TENSOR FASCIAE ANTEBRACHII.	0·978	1·28	0·500	1·11	2·13	1·09	5·95	5·84	5·58	6·54	9·42	10·3	7·85	13·0	11·7	38·4	34·2	29·3

DISSECTION DATA: PIETRAIN

	1.(Q)	2.(O)	3.(L)	4.(E)	5.(N)	6.(I)	7.(F)	8.(P)	9.(K)	10.(S)	11.(C)	12.(R)	13.(H)	14.(J)	15.(M)	16.(G)	17.(D)	18.(B)
M. SUBSCAPULARIS.	2·31	2·50	2·64	3·24	4·75	4·94	10·3	11·9	13·6	18·1	21·6	25·1	47·5	47·5	49·9	98·8	65·2	53·8
M. CORACOBRACHIALIS.	0·465	0·484	0·460	0·451	0·717	1·04	1·69	1·62	2·62	2·55	3·23	4·09	6·57	5·98	6·42	15·0	10·5	15·6
M. BICEPS BRACHII.	1·27	1·41	1·64	2·26	3·64	4·10	5·95	6·62	9·23	10·5	14·1	14·2	26·2	27·5	28·5	54·2	50·0	47·8
M. ARTICULARIS HUMERI.	0·326	1·29	0·434	0·296	0·781	0·297	2·33	1·12	1·23	2·36	2·24	2·91	7·77	5·09	11·4	17·6	26·7	11·4
M. TRICEPS BRACHII. (a) LONG HEAD	11·3	11·6	15·1	19·1	26·8	27·7	55·3	58·9	64·6	87·4	120	122	220	220	210	455	434	421
" " " (b) MEDIAL HEAD.	2·28	1·07	2·25	2·16	3·22	3·98	4·95	6·77	8·24	10·2	15·1	16·4	26·9	27·9	26·3	52·1	40·6	44·4
" " " (c) LATERAL HEAD	3·29	2·69	4·46	4·92	7·09	8·96	15·7	13·8	16·8	25·1	29·4	31·3	43·9	40·5	44·2	95·1	92·3	107
M. BRACHIALIS.	1·98	2·20	3·05	3·57	5·33	5·33	11·2	12·1	12·0	17·3	24·7	27·1	39·6	37·6	34·9	72·4	67·6	71·9
M. EXTENSOR CARPI RADIALIS.	2·54	2·40	3·46	3·22	5·63	5·73	11·6	11·3	13·7	17·3	20·4	24·1	35·0	40·0	36·2	76·8	68·7	81·1
M. ANCONEUS.	0·970	0·756	0·647	0·632	1·08	2·67	1·61	2·77	2·31	3·17	4·80	2·90	7·87	7·26	7·43	13·3	13·8	12·1
M. FLEXOR CARPI ULNARIS.	0·274	0·277	0·265	0·425	0·532	0·456	0·95	1·23	1·07	1·33	1·87	1·67	3·21	2·12	3·01	5·62	5·79	5·04
M. FLEXOR CARPI RADIALIS.	0·510	0·682	0·573	0·757	0·966	1·12	2·09	2·44	2·49	3·35	3·49	4·27	6·32	6·78	7·08	15·4	12·1	12·5
M. PRONATOR TERES.	0·087	0·214	0·060	0·128	0·163	0·137	0·560	0·341	0·418	0·243	0·664	0·664	0·663	1·31	1·36	1·51	1·24	1·41
M. SUPINATOR.	0·101		0·113	*		0·159	0·190	0·238	0·414	0·248	0·492	0·672	0·570	*	0·475	2·35	1·23	2·28
M. FLEXOR DIGITORUM PROFUNDUS + SUPERFICIALIS.	4·05	3·22	3·69	5·18	6·68	7·93	13·9	15·9	22·7	28·5	32·2	38·7	51·7	56·4	59·9	111	116	101
M. EXTENSOR DIGITORUM COMMUNIS.	1·22	0·925	1·60	1·03	1·45	2·00	3·65	4·52	5·23	6·68	8·03	8·75	12·8	13·1	15·5	41·0	23·0	26·2
M. EXTENSOR DIGITORUM LATERALIS.	0·693	0·636	0·864	0·917	1·65	1·57	2·72	2·68	3·98	5·22	4·86	6·11	7·72	7·00	9·05	14·5	16·2	16·7
M. ULNARIS LATERALIS.	0·380	0·282	0·335	0·356	0·718	0·533	1·47	1·30	1·87	1·60	3·13	2·61	3·39	3·54	3·78	7·74	6·12	87·20
M. EXTENSOR CARPI OBLIQUUS.	0·207	0·267	0·298	0·344	0·507	0·571	1·11	0·968	1·45	1·57	1·93	2·18	2·89	2·94	3·07	6·04	6·62	6·21
ABDUCTORS, ADDUCTORS, FLEXORS, OF DIGITS.	0·790	0·296	0·678	0·416	0·799	0·393	1·21	2·15	2·35	3·31	3·75	2·03	3·46	4·02	4·27	5·73	7·78	8·47
(b) HINDLIMB.	*																	
M. TENSOR FASCIAE LATAE.	2·57	2·61	2·73	4·29	5·67	6·63	15·1	14·4	18·1	28·7	31·3	39·1	61·4	68·5	71·5	159	151	160
M. BICEPS FEMORIS.	14·1	19·7	21·6	32·0	41·0	48·5	116	123	149	226	258	293	494	449	474	1,058	1,234	1,175
M. SEMITENDINOSUS.	4·45	5·19	6·10	8·45	13·7	16·2	35·5	31·3	42·7	62·2	76·4	76·4	162	151	165	363	325	318
M. GLUTEUS SUPERFICIALIS.	8·88	3·02	2·06	4·78	8·43	10·2	29·9	27·2	32·3	35·8	61·5	80·9	124	126	99·0	259	316	268
M. GLUTEUS MEDIUS.	4·96	5·83	10·2	10·3	13·8	13·8	25·0	30·8	37·1	78·8	64·8	67·4	118	115	132	319	333	305
M. GLUTEUS ACCESSORIUS.	2·27	1·98	3·32	3·67	5·55	5·97	8·82	12·5	17·7	25·9	25·1	42·2	56·9	64·8	60·5	131	116	145
M. GLUTEUS PROFUNDUS.	1·37	1·13	1·62	1·49	2·92	2·36	4·44	5·46	7·72	12·9	13·3	18·7	30·7	33·7	33·1	57·4	63·0	74·1
M. GRACILIS.	2·89	3·45	4·06	5·86	8·11	7·61	17·5	19·5	21·6	33·5	37·6	44·0	72·8	79·1	90·8	180	177	193
M. SARTORIUS.	0·347	0·486	0·455	0·430	0·558	0·893	2·07	2·46	7·51	4·33	5·05	5·59	6·25	7·63	7·39	16·2	17·4	14·9
M. SEMIMEMBRANOSUS.	8·55	14·0	14·0	26·8	28·9	38·2	100	97·8	119	176	190	211	342	341	333	767	900	776
M. PECTINEUS.	1·21	1·12	1·61	2·21	2·74	3·69	5·96	6·84	7·08	12·0	15·1	18·3	29·6	28·3	33·0	60·0	62·4	78·4
M. ADDUCTOR.	4·01	4·04	5·52	7·26	11·5	14·1	25·3	25·4	34·4	45·8	59·8	63·5	98·4	100	96·7	244	242	256
M. QUADRATUS FEMORIS.	0·614	0·466	0·516	0·436	0·818	0·896	1·22	2·21	2·53	3·03	7·22	4·18	8·50	8·31	7·70	15·4	14·8	14·8
M_m. PSOAS MAJOR et ILIACUS.	5·10	6·15	7·95	9·51	13·0	7·73	38·5	37·0	44·2	62·8	81·1	95·9	141	173	149	380	335	336
M_m. OBTURATOR EXTERNUS et GEMELLI.	1·40	1·18	0·463	2·63	3·34	3·30	7·42	6·89	10·7	12·5	11·5	19·4	37·2	39·1	38·8	88·4	83·7	98·1
M. RECTUS FEMORIS.	5·35	5·76	8·27	9·82	14·8	17·4	32·1	31·1	46·5	59·4	76·3	86·1	150	149	141	324	340	325
M. VASTUS.	8·79	9·46	11·8	16·6	20·9	26·7	49·7	52·8	81·0	109	120	150	252	240	269	542	539	570

DISSECTION DATA: PIETRAIN

	1 (Q)	2 (O)	3 (L)	4 (E)	5 (N)	6 (I)	7 (F)	8 (P)	9 (K)	10 (S)	11 (C)	12 (R)	13 (H)	14 (J)	15 (M)	16 (G)	17 (D)	18 (B)
M. SOLEUS + GASTROCNEMIUS.	4·72	7·00	9·02	11·0	14·1	15·9	36·2	40·7	44·6	72·3	73·9	97·8	154	165	155	291	364	333
M. FLEXOR DIGITORUM SUPERFICIALIS.	1·57	1·31	1·31	1·94	2·95	5·41	7·07	7·63	11·9	13·9	14·3	17·6	32·8	34·0	32·9	57·1	58·1	52·0
M. POPLITEUS.	0·873	0·854	1·03	1·07	1·86	1·88	4·72	3·74	5·74	7·89	8·32	12·1	17·1	17·0	17·7	32·4	34·2	34·3
M. FLEXOR DIGITORUM PROFUNDUS.	1·92	2·36	2·60	3·73	5·71	6·63	14·5	13·4	19·8	36·8	28·7	30·7	55·6	55·0	59·3	109	108	118
M. FIBULARIS LONGUS.	0·578	0·764	0·649	0·600	1·47	1·82	2·64	3·16	4·17	6·45	5·42	7·12	10·4	11·0	13·4	27·9	18·3	25·1
M. FIBULARIS TERTIUS + EXTENSOR DIG. LONGUS.	0·316	1·28	0·780	2·79	3·17	3·36	6·68	5·97	7·90	11·2	12·7	15·3	22·7	23·7	26·3	56·7	44·9	54·6
M. TIBIALIS CRANIALIS.	0·611	0·705	0·769	0·779	1·38	1·68	3·51	3·91	4·58	5·23	6·75	8·63	10·6	12·1	12·3	20·7	22·3	24·8
M. EXTENSOR DIGITI I LONGUS.	0·074	0·111	0·089	0·105	0·250	0·315	0·313	0·346	0·785	0·682	0·636	0·764	1·05	1·07	1·14	3·07	2·92	2·60
M. EXTENSOR DIGITORUM LATERALIS.	0·667	0·568	0·738	0·764	0·890	1·28	3·31	2·72	3·83	5·95	5·24	6·65	10·9	10·9	11·5	24·9	23·3	23·5
M. EXTENSOR DIGITORUM BREVIS.	0·464	0·570	0·548	0·582	1·07	0·946	1·49	2·27	2·56	3·26	3·55	4·85	5·56	6·17	6·09	12·9	9·39	11·1
Mm. INTEROSSEI + ADDUCTORS - DIGITS.	0·208	0·222	0·294	0·236	0·330	0·229	1·42	1·24	0·734	2·59	3·70	3·16	4·08	4·69	4·87	7·41	8·62	8·57
TOTAL MUSCLE MASS (kg) (excluding head muscles) :	0·283	0·292	0·382	0·457	0·687	0·715	1·579	1·658	1·988	2·952	3·599	3·927	6·409	6·492	6·538	14·495	15·033	14·984

BONE WEIGHTS (g)

(a) AXIAL SKELETON.

CERVICAL + THORACIC VERTEBRAE, RIBS + STERNUM	47·8	45·5	45·5	55·1	89·4	67·2	143	169	160	230	339	342	462	460	524	811	773	1,185
LUMBAR + SACRAL VERTEBRAE, + HALF-PELVIS	18·2	16·2	15·9	16·5	29·8	24·6	57·9	70·3	77·1	107	121	130	248	218	253	389	348	415

(b) APPENDICULAR SKELETON.

(i) FORELIMB.

SCAPULA.	4·74	5·25	5·27	7·00	9·65	10·4	17·1	20·6	19·7	36·1	37·4	42·7	72·0	71·7	71·5	130	124	150
HUMERUS.	10·1	8·55	10·6	11·0	16·5	18·8	30·3	34·0	36·7	54·7	65·1	72·2	108	120	124	196	189	228
RADIUS + ULNA.	8·35	7·08	8·13	9·73	13·4	15·2	24·6	28·5	29·3	46·0	50·5	54·9	79·4	83·7	85·4	128	152	159
CARPUS + METACARPUS.	5·46	5·04	5·52	6·32	8·78	11·8	17·5	19·4	22·1	30·9	31·9	38·3	49·2	56·7	55·8	89·0	83·0	89·0

(ii) HINDLIMB.

FEMUR.	10·1	9·19	10·1	13·2	16·9	20·2	34·9	39·3	47·1	69·4	77·6	92·0	130	143	145	201	210	242
PATELLA.	0·596	0·571	0·584	0·915	0·750	1·24	2·02	1·91	3·02	4·41	4·33	5·98	8·18	8·06	8·92	15·4	12·9	14·7
TIBIA + FIBULA.	8·19	7·11	8·34	11·8	14·1	16·5	28·8	31·9	36·8	52·7	55·9	66·7	91·8	99·1	101	148	140	173
TARSUS + METATARSUS.	10·3	9·89	10·7	13·5	15·9	19·0	32·8	38·0	44·0	58·0	57·5	71·4	94·1	100	103	154	153	150
TOTAL BONE WEIGHT (kg)	0·124	0·114	0·123	0·145	0·215	0·205	0·389	0·453	0·475	0·689	0·840	0·916	1·343	1·360	1·472	2·261	2·181	2·806

DISSECTION DATA: PIETRAIN

	1(Q)	2(O)	3(L)	4(E)	5(N)	6(I)	7(F)	8(P)	9(K)	10(S)	11(C)	12(R)	13(H)	14(J)	15(M)	16(G)	17(D)	18(B)
BONE LENGTHS AND WIDTHS (cm)																		
(a) AXIAL SKELETON.																		
THORACIC VERTEBRAE - LENGTH.	12·1	12·0	12·5	13·5	14·9	14·1	19·4	18·0	19·3	24·2	*	26·7	30·0	30·6	31·3	37·2	37·9	38·4
LUMBAR VERTEBRAE - LENGTH.	*	*	5·3	6·6	*	7·3	8·7	*	9·8	11·9	*	*	15·4	16·0	16·8	19·9	19·3	19·5
RIB I - LENGTH.	3·2	3·0	3·2	3·6	4·5	4·1	4·7	5·0	5·9	6·2	*	6·4	9·3	7·2	9·6	10·7	8·2	*
RIB VIII - LENGTH.	6·7	6·1	6·1	6·8	7·4	7·7	8·4	8·8	9·5	9·9	*	10·2	13·0	14·2	14·3	17·1	15·3	*
(b) APPENDICULAR SKELETON.																		
(i) FORELIMB.																		
SCAPULA - LENGTH.	5·7	5·0	5·3	5·4	6·0	7·7	7·5	7·4	8·4	8·6	9·8	9·5	12·4	12·4	12·5	15·0	17·5	16·9
HUMERUS - LENGTH	5·9	5·3	6·9	5·8	6·8	7·1	7·9	8·0	8·8	9·3	10·3	10·4	12·4	12·6	13·0	15·4	15·2	16·2
- WIDTH.	0·8	0·8	0·9	0·8	1·0	1·0	1·1	1·3	1·3	1·6	1·6	1·7	2·1	2·0	2·0	2·5	2·6	2·5
RADIUS - LENGTH	6·4	6·0	6·0	6·3	7·3	7·0	8·8	9·3	9·8	11·1	11·6	12·2	14·2	14·6	14·4	16·4	16·3	17·2
- WIDTH.	1·3	1·2	1·3	1·5	1·6	1·5	1·8	2·1	2·0	2·5	2·5	2·8	3·0	3·1	2·9	3·2	3·5	3·5
METACARPUS IV - LENGTH	2·6	2·3	2·7	2·7	3·0	3·8	3·6	4·0	4·1	4·5	4·7	6·0	5·8	5·8	5·7	7·6	8·0	6·7
- WIDTH.	0·5	0·6	0·8	0·7	0·6	0·9	0·9	1·1	1·1	1·2	1·4	1·3	1·4	1·5	1·4	1·6	1·7	1·7
(ii) HINDLIMB.																		
FEMUR - LENGTH.	5·9	5·5	5·6	6·0	6·4	7·0	8·3	8·2	9·4	10·6	11·3	11·5	13·9	14·6	15·0	16·6	17·2	18·6
- WIDTH.	0·8	0·7	0·7	0·9	1·0	1·0	1·2	1·3	1·4	1·6	1·5	1·7	2·0	2·0	1·9	2·2	2·2	2·3
TIBIA - LENGTH.	5·5	5·1	5·1	5·6	6·1	6·4	7·9	7·8	8·5	10·0	10·5	11·0	13·3	13·8	13·5	15·9	15·4	16·8
- WIDTH.	0·9	0·7	0·8	0·8	0·9	1·1	1·2	1·4	1·5	1·7	1·6	1·8	1·8	1·9	1·9	2·2	2·2	2·1
METATARSUS IV - LENGTH	3·0	3·4	3·0	3·0	3	3·7	5·3	5·3	5·0	5·1	5·2	5·7	6·7	6·7	6·8	9·5	9·1	10·6
- WIDTH.	0·6	0·7	0·9	0·8	0·8	0·8	1·0	1·0	1·0	1·2	1·1	1·2	1·2	1·2	1·3	1·5	1·6	1·6
FAT WEIGHTS.																		
(a) ABDOMINAL (g)	0·158	1·20	1·26	8·48	5·69	5·71	23·1	18·7	5·86	27·5	73·5	29·6	115	169	135	432	234	250
(b) INTERMUSCULAR (g.)	Zero.	18·0	12·4	44·9	66·7	56·9	112	169	132	172	257	243	679	715	860	1,806	1,596	1,450
(c) SUBCUTANEOUS. (g.)	Zero.	45·5	36·2	74·4	118	118	185	373	206	475	717	355	1,074	1,657	1,529	3,089	3,882	2,518
TOTAL FAT WEIGHT (kg.)	Zero.	0·065	0·050	0·128	0·191	0·181	0·320	0·561	0·342	0·675	1·048	0·628	1·868	2·541	2·524	5·327	5·712	4·218
TENDON WEIGHT. (g.)	7·44	7·63	14·0	10·5	17·5	18·2	37·5	50·1	49·6	71·5	63·9	76·3	209	131	163	243	221	278
INTEGUMENT WEIGHT (g.) (includes digits and tail)	80·5	66·8	86·9	113	119	134	291	261	295	425	379	617	638	708	777	1,105	1,421	1,383

* INDICATES ABSENCE OF PART OF MUSCLE, OR NON-AVAILABILITY OF RESULT.

DISSECTION DATA: LARGE WHITE

	1 (CC)	2 (CK)	3 (CH)	4 (CA)	5 (CS)	6 (CV)	7 (CI)	8 (CL)	9 (CG)	10 (CA)	11 (CB)	12 (CN)	13 (CQ)	14 (CF)	15 (CE)	16 (CD)	17 (CO)	18 (CP)
LIVE WEIGHT. (Kg).	1·27	3·72	3·69	3·98	4·16	7·69	8·11	7·33	9·46	13·0	13·5	15·0	(32·0)	(32·0)	(32·0)	(64·0)	(64·0)	(64·0)
AGE AT SLAUGHTER. (DAYS).	2	12	12	10	13	53	48	19	49	56	56	56	101	100	100	158	214	170
DATE OF BIRTH.	9·8·71	8·8·71	11·8·71	27·8·71	17·8·71	3·7·71	6·7·71	18·7·71	5·7·71	16·6·71	16·6·71	5·7·71	28·5·71	8·5·71	8·6·71	11·3·71	4·2·71	20·3·71
COLD CARCASS WEIGHT. (Kg)	1·02	3·02	3·05	3·21	3·59	5·76	5·89	6·18	7·14	9·58	10·3	11·2	19·0	21·1	22·0	44·8	46·0	46·5
HALF CARCASS WEIGHT. (Kg)	0·495	1·50	1·44	1·57	1·64	2·74	2·89	3·18	3·63	4·75	5·13	5·54	9·23	10·8	11·1	22·5	21·6	22·3
WEIGHT OF VISCERA																		
HEART. (g).	10·0	28·0	22·7	30·0	23·0	45·0	47·0	41·0	50·0	62·0	73·0	74·0	136	144	146	210	265	230
LUNGS. (g)	21·0	73·0	59·0	69·0	54·0	105	113	92·0	115	165	164	160	358	363	425	948	850	875
LIVER. (g).	36·0	111	106	133	107	266	366	197	321	368	367	475	1180	980	374	435	1610	1200
SPLEEN. (g).	2·00	9·00	10·8	10·0	10·0	15·0	29·0	20·0	16·4	20·0	24·0	33·0	66·0	55·0	43·0	75·0	134	82·0
STOMACH. (g).	7·50	23·0	22·0	32·0	25·0	78·0	74·0	42·0	90·4	125	140	186	362	405	326	462	586	602
SMALL INTESTINE. (g).	42·0	111	102	140	112	333	505	166	442	650	630	722	1180	1120	1140	1040	1600	1370
LARGE INTESTINE. (g).	25·0	52·0	58·0	50·0	50·0	166	224	76·0	167	270	324	325	782	700	644	950	1240	926
GUT CONTENT. (g).	38·5	79·0	98·5	128	117	543	454	758	512	627	823	1510	3120	3424	2462	5180	3990	2230
UTERINE HORNS + OVARIES. (g).	1·00	5·00	1·50	1·92	6·00	4·14	5·00	8·00	5·00	2·50	2·60	4·92	26·0	10·0	*	58·0	29·0	67·5
KIDNEY. (g).	4·14	11·4	13·6	11·1	11·5	17·1	24·9	17·2	22·6	27·2	29·3	35·7	69·8	59·8	57·0	95·0	112	104
ADRENAL GLAND. (g).	0·090	0·200	0·154	0·200	0·200	0·292	0·433	0·220	0·355	0·440	0·550	0·528	0·723	0·589	0·654	1·30	1·19	1·87
HINDQUARTER WEIGHT. (Kg).	0·162	0·526	0·498	0·525	0·560	1·00	1·00	1·07	1·24	1·79	1·88	2·06	3·43	8·02	4·30	9·07	8·39	8·62
FOREQUARTER WEIGHT. (Kg).	0·331	0·925	0·903	1·00	1·03	1·65	1·77	2·03	2·26	2·82	3·08	3·30	5·40	6·32	6·39	13·1	12·4	13·0
AXIAL SKELETON MUSCLE WEIGHTS																		
a) HEAD (g).																		
M. LEVATOR LABII MAXILLARIS.	0·560	1·17	1·12	0·693	1·56	2·34	2·79	2·62	2·51	3·80	3·92	4·13	6·32	6·46	7·67	15·5	17·0	15·9
M. LATERALIS NASI.	0·203	0·580	0·754	1·25	0·704	1·20	1·12	1·24	1·62	1·27	1·61	1·13	2·39	2·25	2·64	5·17	6·59	6·50
M. DEPRESSOR LABII MAXILLARIS.	0·486	0·736	0·720	1·09	0·808	1·64	1·38	1·83	0·880	2·18	2·71	2·86	3·26	5·07	6·15	7·81	11·4	9·19
M. DIGASTRICUS.	0·369	0·694	0·762	0·852	0·870	1·25	1·36	1·42	1·51	2·21	2·63	2·10	3·84	3·12	3·44	8·70	8·07	8·03
M. TEMPORALIS.	1·29	4·03	3·14	3·41	4·40	4·90	6·53	5·02	6·65	10·7	11·0	10·6	26·9	20·3	20·2	48·5	48·6	39·5
M. PTERYGOIDEUS.	1·26	2·48	2·06	3·89	3·47	6·50	6·40	3·78	8·06	8·37	10·5	11·4	25·9	16·5	16·1	40·7	32·6	31·1
M. MASSETER.	1·91	4·64	4·47	3·86	5·28	8·95	10·9	8·44	14·3	19·3	19·3	17·7	30·6	56·6	37·9	81·1	66·0	79·5
b) NECK AND THORAX (g)																		
M. SCALENUS.	1·38	3·50	3·42	2·96	3·87	3·76	8·50	4·78	8·64	11·2	11·0	11·0	18·4	31·5	7·84	46·8	68·1	63·1
M. ILIOCOSTALIS THORACIS.	0·680	3·14	3·20	3·26	3·63	7·00	9·60	6·78	9·64	10·8	13·8	14·6	29·0	32·1	50·9	63·4	81·1	64·0
M. LONGISSIMUS THORACIS.	7·93	33·1	29·2	32·0	37·9	58·9	62·4	80·9	79·2	122	127	127	218	257	286	648	651	610
M. SPLENIUS CERVICIS.	2·36	7·43	7·27	9·04	6·51	11·9	20·4	17·0	16·3	19·8	26·6	26·1	42·1	50·0	60·8	110	110	99·6
M. SEMISPINALIS - BIVENTER CERVICIS.	2·46	8·18	4·56	11·0	4·47	7·82	14·7	18·7	15·5	22·5	24·3	22·6	40·2	52·1	63·9	120	75·9	85·0
M. " - COMPLEXUS.	2·07	4·17	6·61	6·51	6·70	9·76	13·8	9·35	13·3	21·4	20·8	14·3	32·5	60·0	55·6	88·3	72·1	69·2
M. SPINALIS.	1·19	7·63	4·73	8·17	6·50	12·0	19·8	18·0	16·7	21·2	30·9	25·7	34·4	57·1	31·1	114	147	136
M. STERNOMASTOIDEUS.	1·45	1·29	2·30	0·650	1·92	4·44	4·46	4·60	4·96	6·65	7·50	7·16	8·91	15·7	16·1	35·6	26·8	32·2
M. STERNOHYOIDEUS + STERNOTHYROIDEUS.	0·305		1·96	*	1·60	*	4·66	4·81	1·24	7·54	14·5	2·80	11·8	31·2	25·6	38·9	58·6	41·2
DEEP NECK MUSCLES.	4·11	21·0	12·7	23·7	15·9	38·0	46·0	40·2	38·0	58·9	48·0	73·3	117	172	47·0	234	317	328
MM. INTERCOSTALES EXTERNI ET INTERNI.	11·1	26·2	33·3	23·1	28·6	48·7	56·8	57·0	66·0	79·2	92·0	103	186	213	296	469	453	497

DISSECTION DATA: LARGE WHITE

	1(CC)	2(CK)	3(CH)	4(CR)	5(CJ)	6(CM)	7(CI)	8(CL)	9(CG)	10(CN)	11(CP)	12(CN)	13(CQ)	14(CF)	15(CE)	16(CD)	17(CO)	18(CF)
c) LUMBO-SACRAL REGION (g)																		
M. PSOAS MINOR.	0·162	0·950	1·00	2·45	1·05	1·64	1·72	1·75	2·89	5·37	4·20	2·86	7·89	9·50	8·12	14·6	17·2	12·2
M. ILIOCOSTALIS LUMBORUM.	0·009	*	0·230	0·318	*	0·260	0·146	0·642	0·358	0·446	0·822	0·790	1·37	1·90	3·40	3·62	3·21	4·02
M. LONGISSIMUS LUMBORUM.	4·70	20·2	20·3	17·4	18·9	35·9	33·8	41·8	46·0	78·3	80·6	82·4	158	199	200	492	440	380
DEEP LUMBAR MUSCLES.	0·937	5·32	1·82	3·10	4·35	11·4	15·0	15·6	25·0	31·5	22·8	32·3	31·7	61·8	67·0	114	164	217
d) ABDOMINAL MUSCLES (g)																		
DIAPHRAGMA.	2·09	4·81	4·97	8·43	4·86	14·1	13·9	10·3	20·6	17·4	28·2	16·6	54·7	54·3	59·5	117	42·0	83·1
M. TRANSVERSUS ABDOMINIS.	4·18	7·89	8·05	9·14	8·32	16·8	23·8	14·4	23·7	35·3	36·5	35·4	73·1	71·4	85·0	188	164	183
M. RECTUS ABDOMINIS.	3·06	9·88	9·05	9·84	10·4	19·8	28·9	17·5	24·9	39·0	39·3	45·2	73·1	83·5	89·3	193	186	159
M. OBLIQUUS EXTERNUS ABDOMINIS.	*	6·13	4·06	4·87	5·19	10·9	12·3	11·0	12·55	23·5	19·8	24·2	34·5	40·4	46·6	115	*	70·3
M. OBLIQUUS INTERNUS ABDOMINIS.	0·415	5·79	5·21	6·34	5·65	11·3	13·0	8·52	15·3	18·9	22·3	26·2	47·0	55·8	62·4	123	124	120
e) CUTANEOUS MUSCLES.																		
M. CUTANEOUS TRUNCI.	*	7·44	4·54	5·78	5·08	14·9	17·4	10·9	12·2	21·0	12·7	23·5	34·8	57·0	50·3	132	136	95·0
M. CUTANEOUS OMOBRACHIALIS + COLLI	2·77	14·1	8·56	15·5	18·6	24·4	23·1	21·9	38·7	38·5	64·9	51·2	102	86·2	95·0	313	278	230
APPENDICULAR SKELETON MUSCLE WEIGHTS																		
a) FORELIMB. (g)																		
M. TRAPEZIUS.	3·50	7·38	3·79	8·31	8·66	9·25	14·2	12·2	18·7	21·3	31·6	24·0	43·7	48·4	51·8	125	117	113
M. BRACHIOCEPHALICUS.	2·31	8·00	8·13	9·43	8·75	17·0	16·4	15·5	17·4	24·2	29·9	28·4	46·5	49·9	51·6	132	126	104
M. OMOTRANSVERSARIUS.	0·311	0·594	0·782	0·850	0·572	1·26	1·65	2·04	1·72	3·40	3·63	2·29	4·01	4·44	4·91	11·1	12·5	124
M. RHOMBOIDEUS.	2·94	5·63	8·11	6·64	6·97	11·5	10·7	11·6	17·3	23·8	28·0	29·9	31·7	26 2	47·3	81·3	107	81·1
M. PECTORALIS SUPERFICIALIS.	2·54	5·15	7·62	5·89	6·43	9·35	12·9	12·0	13·0	16·7	21·3	24·0	27·4	39·2	40·6	90·0	87·4	128
M. PECTORALIS ASCENDENS.	5·11	16·8	10·2	19·8	18·3	31·7	29·7	39·7	48·7	67·1	64·8	69·9	97·3	122	129	315	307	328
M. PECTORALIS CLEIDOSCAPURIS.	3·21	8·20	9·31	8·73	10·4	19·2	18·0	18·9	25·2	35·5	35·6	39·4	57·8	65·6	72·4	168	168	168
M. LATISSIMUS DORSI.	6·26	14·0	15·7	18·2	17·5	24·4	30·7	26·9	34·8	47·5	52·9	58·8	73·6	105	89·8	263	256	219
M. SERRATUS VENTRALIS.	7·65	23·7	26·0	25·0	24·3	38·8	61·7	41·8	46·8	78·7	75·0	91·0	155	164	186	438	385	397
M. SERRATUS DORSALIS.	3·09	1·55	1·76	1·95	3·14	3·19	2·27	4·08	2·57	5·92	4·27	2·96	16·1	6·48	6·35	14·3	58·1	53·1
M. TERES MAJOR.	1·53	4·11	4·85	4·46	4·47	7·60	8·97	7·94	10·0	12·1	13·6	16·3	23·0	27·1	25·7	60·0	59·6	56·2
M. TERES MINOR.	0·455	1·11	1·03	1·08	1·07	2·37	2·50	2·47	2·50	4·11	4·59	4·24	6·94	14·6	4·57	23·8	22·4	21·4
M. SUPRASPINATUS.	5·28	15·3	19·1	14·6	16·7	26·9	29·7	31·2	33·8	51·3	49·2	59·0	98·5	112	124	296	239	209
M. INFRASPINATUS.	3·87	10·3	12·3	12·0	11·7	20·6	24·3	20·1	26·3	39·5	40·6	44·4	82·1	78·1	105	212	206	205
M. DELTOIDEUS.	0·955	2·81	3·05	3·02	3·41	4·60	4·62	5·29	6·45	7·69	8·33	9·48	16·8	20·6	19·6	46·2	44·4	38·7
M. TENSOR FASCIAE ANTEBRACHII.	0·622	1·96	2·20	2·13	1·96	3·59	3·57	3·61	4·45	6·66	8·82	6·87	11·0	11·4	10·2	29·3	31·9	21·9
M. SUBSCAPULARIS.	1·78	4·25	5·14	4·80	4·86	8·37	10·5	8·61	11·8	16·8	18·0	18·4	32·6	35·4	34·4	79·4	77·3	76·2
M. CORACOBRACHIALIS.	0·300	0·540	0·724	0·810	0·761	1·36	1·50	1·42	1·53	2·66	2·60	2·79	4·36	4·04	5·27	12·3	12·2	10·2
M. BICEPS BRACHII.	0·930	2·66	3·21	3·03	3·34	5·35	5·21	6·12	6·50	9·41	9·54	9·84	16·4	19·1	19·4	44·2	40·2	46·8
M. ARTICULARIS HUMERI.	0·195	0·465	0·445	0·761	0·447	1·07	0·604	0·771	0·908	1·66	1·57	2·23	5·34	4·20	4·98	7·55	7·19	6·44
M. TRICEPS BRACHII. a) LONG HEAD.	6·75	20·3	21·3	21·0	24·7	33·5	39·4	43·1	62·8	71·2	70·1	80·2	124	146	152	351	316	296
" b) MEDIAL HEAD.	1·20	2·49	2·99	2·70	2·40	5·50	6·18	5·56	6·47	9·70	8·95	11·9	22·5	21·2	25·0	49·3	41·4	41·0
" c) LATERAL HEAD.	2·18	6·31	7·68	7·13	6·58	10·1	11·9	12·4	15·3	20·4	18·7	22·6	34·4	43·3	43·3	83·0	79·2	82·0
M. BRACHIALIS.	1·67	4·81	5·22	4·82	4·49	8·00	8·21	7·84	10·3	12·9	14·2	15·5	24·8	27·4	30·0	64·4	57·9	57·5

DISSECTION DATA: LARGE WHITE

	1(cc)	2(ck)	3(ch)	4(cr)	5(cj)	6(cm	7(ci)	8(cl)	9(cg)	10(ca)	11(cb)	12(cn)	13(cq)	14(cf)	15(ce)	16(cd)	17(co)	18(cp)
M. Extensor carpi radialis.	1·63	4·58	5·39	4·29	5·13	7·91	8·17	9·12	9·74	14·4	13·6	17·7	26·7	27·7	34·8	67·7	59·7	62·0
M. Anconeus.	0·585	1·23	1·37	1·27	1·26	2·23	2·37	1·79	5·23	3·80	4·53	3·73	7·85	6·38	7·72	17·3	16·9	14·5
M. Flexor carpi ulnaris.	0·150	0·410	0·518	0·570	0·600	0·800	0·687	0·800	0·939	1·39	1·11	1·80	2·46	4·30	3·21	6·12	5·42	6·14
M. Flexor carpi radialis	0·220	1·00	0·832	1·06	1·08	1·45	1·84	1·66	1·82	2·84	2·57	3·29	5·41	5·22	6·65	14·5	11·4	10·0
M. Pronator teres.	0·003	0·187	0·140	0·190	0·218	0·268	0·175	0·222	0·400	0·003	0·263	0·402	0·322	1·07	0·589	1·69	0·439	1·94
M. Supinator.	0·240	0·055	0·178	0·120	0·315	0·479	0·177	0·475	0·240	0·342	0·259	0·162	*	0·650	0·769	1·51	1·78	2·50
M. Flexor Digitorum Profundus + Superficialis	2·34	5·84	6·98	7·10	8·39	14·0	13·8	13·9	12·4	26·1	25·0	25·6	35·8	54·4	11·6	98·4	97·4	103
M. Extensor Digitorum Communis.	0·640	1·47	1·87	2·08	1·98	3·63	2·80	2·98	5·47	4·67	5·29	5·46	10·0	10·6	11·1	25·4	23·1	22·3
M. Extensor Digitorum Lateralis.	0·480	1·47	1·75	1·65	1·82	2·32	2·15	2·56	0·984	3·90	4·27	4·12	6·94	8·22	8·66	15·2	13·6	18·2
M. Ulnaris Lateralis.	0·210	0·877	1·07	0·811	1·02	1·24	0·917	1·44	1·72	1·69	1·76	1·96	3·89	2·87	4·52	6·65	8·08	9·23
M. Extensor carpi obliquus.	0·125	0·364	1·10	0·528	0·411	0·710	0·748	0·555	0·937	1·11	0·959	1·42	1·75	2·50	2·91	4·26	3·69	4·82
Abductors, Adductors + Flexors of Digits.	0·008	0·720	0·200	1·57	0·600	2·57	1·10	1·10	2·34	2·13	2·46	5·06	3·10	2·80	3·58	5·05	8·66	10·7
M. Flexor Digitorum V	0·077	0·129	0·085	0·125	0·123	0·233	0·237	0·133	0·120	0·206	0·143	0·307	3·82	0·255	0·358	0·501	0·323	0·767
B Hindlimb.																		
M. Tensor Fasciae Latae.	1·85	5·40	4·58	4·44	4·86	8·85	11·1	9·86	14·2	19·1	21·1	20·9	28·6	37·6	38·7	108	99·4	87·2
M. Biceps Femoris.	9·85	43·2	40·7	42·1	41·5	70·7	72·2	78·8	97·0	149	151	145	286	325	342	905	782	757
M. Semitendinosus.	4·22	11·3	11·0	11·6	12·8	21·0	25·1	23·3	26·4	38·9	48·5	47·2	81·6	91·5	105	299	222	230
M. Gluteus Superficialis.	2·05	8·18	9·25	9·57	7·28	17·3	16·0	16·9	17·3	35·6	38·7	39·0	74·5	101	107	189	191	186
M. Gluteus Accessorius	1·93	4·63	4·58	3·97	4·91	7·70	10·3	9·04	11·4	20·0	16·8	19·4	39·0	41·2	43·2	95·0	99·2	79·7
M. Gluteus Medius.	2·69	10·6	8·68	7·88	10·1	17·1	18·2	20·8	29·4	31·3	35·9	44·0	66·7	70·0	62·7	218	190	177
M. Gluteus Profundus.	1·07	2·54	2·80	0·906	2·91	5·25	6·62	5·38	6·30	9·65	11·1	11·1	21·2	21·9	23·2	50·0	52·9	60·6
M. Gracilis.	2·02	6·59	6·45	6·43	6·92	11·2	12·9	13·6	15·3	23·1	25·4	24·5	41·5	50·8	56·0	135	100	132
M. Sartorius.	0·248	1·06	0·694	0·636	1·20	1·36	1·30	1·33	1·70	2·81	2·55	2·95	5·91	5·12	8·91	14·0	7·90	12·1
M. Semimembranosus.	7·26	28·3	27·4	26·5	27·5	54·8	54·6	61·6	69·4	114	117	123	187	231	258	595	502	475
M. Pectineus.	0·924	2·73	2·38	2·33	2·78	4·72	5·11	4·92	6·67	9·18	10·0	9·66	18·7	18·7	22·1	49·5	46·4	47·9
M. Adductor.	1·55	8·90	5·07	10·3	9·33	17·3	20·0	20·6	22·1	39·1	36·7	39·5	62·6	73·0	91·4	199	103	167
M. Quadratus Femoris.	0·293	0·450	0·600	0·406	0·600	1·32	1·48	0·950	1·44	1·91	1·86	2·30	3·98	4·75	4·79	12·7	15·3	11·1
Mm. Psoas Major et Iliacus.	4·91	15·0	13·9	14·9	15·1	25·0	30·0	26·3	32·2	48·4	57·4	51·6	104	102	100	262	270	228
Mm. Obturator Externus et Gemellus.	1·04	3·20	3·37	4·18	2·78	6·81	5·97	8·02	9·38	13·2	15·6	12·1	26·5	30·8	29·6	71·4	67·5	65·6
M. Rectus Femoris.	4·27	13·7	12·9	13·8	14·3	25·3	22·6	27·0	32·4	49·0	52·3	51·8	87·5	102	104	227	225	236
M. Vasti.	6·16	19·1	20·6	23·1	21·6	37·5	41·1	41·8	45·7	82·2	78·5	89·2	137	162	174	376	335	413
M. Soleus et Gastrocnemius.	3·83	15·0	13·9	12·1	15·2	27·2	23·7	33·7	34·3	54·8	52·3	53·6	94·4	114	112	268	234	262
M. Flexor Digitorum Superficialis.	0·799	2·31	2·55	2·79	2·92	4·91	5·20	5·47	6·74	9·27	9·05	11·9	16·6	22·4	21·5	41·2	35·1	38·6
M. Popliteus.	0·922	1·78	1·81	1·74	2·10	3·89	3·65	3·36	4·19	6·85	7·21	6·95	9·80	11·3	12·7	28·2	26·4	28·9
M. Flexor Digitorum Profundus.	1·26	1·37	5·26	5·53	5·68	11·3	11·2	9·93	12·8	21·1	20·4	22·8	33·8	40·0	44·1	93·6	77·7	88·2
M. Fibularis Longus.	0·396	5·45	1·30	1·52	1·25	2·24	2·38	2·28	2·81	4·52	4·68	4·84	6·95	6·89	9·21	20·1	19·1	20·7
Mm. Fibularis Tertius et Ext. Dig. Longus.	0·776	2·34	2·51	2·47	2·85	4·26	5·13	5·50	5·45	8·91	8·56	9·13	14·9	19·8	19·2	42·3	40·5	45·2
M. Tibialis Cranialis.	0·420	1·40	1·05	1·41	1·50	2·35	2·68	2·48	2·68	4·36	4·29	4·50	8·48	8·45	7·95	19·2	16·9	19·9
M. Extensor Digiti I Longus.	0·057	0·172	0·298	0·174	0·274	0·244	0·238	0·216	0·346	0·452	0·710	0·680	0·958	0·924	0·940	2·04	1·55	2·20
M. Extensor Digitorum Lateralis.	0·448	1·17	1·16	1·42	1·21	2·16	2·11	2·21	2·65	4·69	4·88	4·11	7·29	8·74	9·76	19·0	17·9	23·1
M. Extensor Digitorum Brevis.	0·317	1·20	0·778	0·800	0·934	1·71	1·28	1·67	1·55	2·63	2·70	3·14	4·93	6·35	6·03	12·5	8·41	8·60
Mm. Interossei et Adductors of Digits.	0·244	0·970	0·691	0·720	0·970	1·38	1·48	1·36	1·24	2·29	3·33	2·97	4·40	3·48	3·85	3·83	8·80	8·21

DISSECTION DATA: LARGE WHITE

	1(CC)	2(CK)	3(CH)	4(CR)	5(CJ)	6(CM)	7(CI)	8(CL)	9(CG)	10(CA)	11(CB)	12(CN)	13(CQ)	14(CF)	15(CE)	16(CD)	17(CO)	18(CP)
TOTAL MUSCLE MASS (Kg) (EXCLUDING HEAD MUSCLES)	·186	·612	·612	·632	·647	1·12	1·26	1·25	1·47	2·18	2·29	2·40	4·05	4·77	4·86	11·5	10·7	10·7
BONE WEIGHTS. (g).																		
a) AXIAL SKELETON.																		
CERVICAL + THORACIC VERTEBRAE. RIBS + STERNEBRAE	27·0	96·0	76·0	86·0	82·0	160	152	188	225	234	305	333	424	520	508	929	1080	892
LUMBAR + SACRAL VERTEBRAE. HALF PELVIS.	12·8	31·5	41·7	26·3	29·2	73·2	60·7	51·2	128	122	126	140	162	246	292	398	443	445
b) APPENDICULAR SKELETON.																		
i) FORELIMB																		
SCAPULA.	3·35	9·83	10·7	10·2	12·4	20·1	19·5	19·9	26·4	34·5	34·6	42·2	65·1	82·0	72·2	168	143	129
HUMERUS.	7·58	16·6	19·1	17·5	18·8	38·8	38·0	29·8	44·7	64·3	67·3	70·9	110	130	126	231	229	113
RADIUS & ULNA.	6·12	13·3	15·3	15·5	15·9	29·6	28·9	24·9	35·5	48·6	52·2	59·3	84·7	99·0	97·0	174	168	156
CARPUS & METACARPUS.	4·64	10·8	11·8	12·1	13·4	21·1	23·0	18·8	28·5	35·4	38·4	39·2	61·7	70·1	65·9	118	113	95·0
ii) HINDLIMB.																		
FEMUR.	7·05	18·0	18·8	20·5	19·8	44·4	43·9	32·4	48·6	72·8	75·3	86·0	128	136	134	246	247	238
PATELLA.	0·454	0·964	1·19	1·00	0·945	2·39	2·73	1·53	2·59	3·75	4·00	5·45	8·88	8·26	8·70	16·3	16·8	16·2
TIBIA & FIBULA.	6·24	14·8	14·5	18·1	16·1	33·4	31·2	26·7	36·2	51·9	55·7	62·7	95·0	111	100	190	179	164
TARSUS & METATARSUS.	6·59	18·5	17·8	22·1	20·5	42·4	37·2	31·6	42·8	63·7	62·0	66·4	105	114	105	184	187	174
TOTAL BONE WEIGHT. (kg).	0·082	0·230	0·218	0·224	0·229	0·465	0·437	0·424	0·618	0·731	0·821	0·905	1·24	1·52	1·51	2·65	2·81	2·42
BONE DIMENSIONS. (cm).																		
THORACIC VERTEBRAE - LENGTH.	11·0	16·6	15·8	17·0	16·6	22·8	23·6	21·6	23·6	26·3	28·4	29·1	34·5	36·5	35·6	34·8	43·5	41·9
LUMBAR VERTEBRAE - LENGTH.	5·00	7·10	6·80	6·90	7·50	10·1	10·0	8·90	11·2	12·5	13·2	13·0	16·8	16·6	17·0	19·9	20·7	21·0
RIB No. I.	3·10	3·70	4·50	*	3·90	5·10	5·50	5·10	6·50	4·90	7·60	7·10	8·00	9·00	8·00	10·5	11·0	10·7
RIB No. VIII.	5·10	7·40	7·60	7·70	7·50	10·2	9·20	9·40	9·90	11·20	11·3	10·7	13·7	14·7	14·4	17·4	16·2	16·1
i) FORELIMB.																		
SCAPULA - LENGTH.	4·80	7·10	7·80	6·00	7·50	10·0	9·50	8·70	10·0	10·6	10·5	12·8	14·9	14·2	12·9	16·8	17·9	18·5
WIDTH.	1·90	1·00	1·00	1·10	1·20	1·20	1·20	1·15	1·20	1·60	1·40	1·70	1·80	2·00	1·90	2·40	2·30	2·2
HUMERUS - LENGTH.	5·30	6·80	6·80	7·30	6·70	9·00	8·70	8·90	8·80	11·4	11·4	11·2	12·8	12·8	14·0	15·2	17·1	16·7
WIDTH.	1·60	0·700	0·750	1·05	0·750	0·950	1·00	0·900	1·10	1·50	1·70	1·60	1·30	1·50	2·20	1·90	1·80	2·60

DISSECTION DATA: LARGE WHITE

	1 (CC)	2 (CK)	3 (CH)	4 (CQ)	5 (CJ)	6 (CM)	7 (CI)	8 (CL)	9 (CG)	10 (CA)	11 (CB)	12 (CN)	13 (CQ)	14 (CE)	15 (CE)	16 (CD)	17 (CO)	18 (CF)
RADIUS – LENGTH.	3·50	4·70	4·70	4·60	4·70	6·30	6·10	5·60	6·50	12·1	12·7	7·40	9·20	9·70	15·2	11·1	11·7	11·7
WIDTH.	0·700	0·900	0·950	0·850	1·00	1·10	1·20	1·05	1·30	2·20	2·30	1·50	1·60	1·80	3·10	2·30	2·10	2·00
METACARPUS IV – LENGTH.	2·20	3·30	3·20	3·20	3·20	4·30	4·00	3·60	4·50	4·60	5·00	5·00	5·96	5·80	6·10	6·70	6·70	6·70
WIDTH.	0·800	0·750	1·00	0·800	1·05	1·15	1·10	0·950	1·30	1·50	1·40	1·20	1·40	1·50	1·50	1·80	1·60	1·40
ii) HINDLIMB																		
FEMUR – LENGTH.	5·40	7·20	7·10	7·00	7·30	9·90	9·70	8·40	10·5	11·9	11·8	12·1	14·8	14·2	14·6	19·3	18·5	18·1
WIDTH.	0·620	0·900	0·800	0·860	0·850	1·20	1·10	1·10	1·20	1·30	1·20	1·30	1·55	1·20	1·70	2·10	2·20	2·10
TIBIA – LENGTH.	5·10	6·80	6·80	6·20	6·40	9·20	8·60	8·30	8·70	10·5	11·0	11·0	13·9	13·5	13·3	15·8	17·0	16·9
WIDTH.	0·800	0·950	0·900	0·900	1·00	1·20	1·20	1·10	1·25	1·40	1·50	1·35	1·20	1·70	1·60	2·10	1·60	1·50
METATARSUS IV – LENGTH	2·30	3·40	4·50	3·60	3·40	4·45	4·60	4·60	5·80	5·60	6·00	5·20	7·00	8·20	8·00	7·70	8·35	8·00
WIDTH.	0·600	0·800	0·900	0·800	0·900	0·950	1·20	1·00	1·00	1·50	1·20	1·00	1·20	1·40	1·40	1·50	1·70	1·50
FAT WEIGHTS																		
a) ABDOMINAL. (g)	0·000	7·00	2·60	5·20	10·0	17·0	14·0	25·0	36·0	25·0	31·0	23·5	117	120	124	282	215	477
b) INTERMUSCULAR. (g)	0·030	68·0	39·0	56·2	45·9	104	84·0	144	129	125	111	187	383	483	433	1030	1100	1210
c) SUBCUTANEOUS. (g)	0·000	149	135	141	200	234	236	450	309	417	498	570	1400	1190	1680	3260	2310	3430
TOTAL FAT WEIGHT (Kg)	0·000	0·22[illegible]	[illegible]·177	0·202	0·256	0·338	0·334	0·619	0·474	0·567	0·640	0·781	1·90	1·80	2·24	4·57	3·42	5·12
TOTAL MISCELLANEOUS (Kg) (including hals head, tail, digits, tendon, integument & scrap.	0·192	0·392	0·364	0·448	0·438	0·737	0·727	0·797	0·998	1·15	1·18	1·30	1·87	2·29	2·21	3·84	3·86	3·92

* – INDICATES ABSENCE OF PART OF MUSCLE OR NON AVAILABILITY OF RESULT

J. Anat. (1972), **112**, 1, pp. 41–60
With 20 *figures*
Printed in Great Britain

Histochemical fibre types in the mammalian diaphragm

A. S. DAVIES AND H. M. GUNN

Department of Anatomy, Royal (Dick) School of Veterinary Studies, Edinburgh

(*Accepted* 3 *March* 1972)

INTRODUCTION

In 1873, Ranvier showed that differences in the colour of femoral muscles of the rabbit could be related to differences in speed of contraction. This relationship has rarely been questioned, even though biochemical advances have shown that the redness is due to iron-containing pigments involved in oxygen and electron transport, and there is no reason to suppose that variations in the structure and function of the contractile myofilaments need be reflected in changes in the colour of the muscle. The advent of histochemical methods which reveal, in single fibres, the enzymes concerned with the release of energy and its connexion to a contractile force, has made the characterization of fibre types in muscle progressively more difficult.

Different levels of succinate dehydrogenase (SDHase) activity in individual muscle fibres were demonstrated by Padykula (1952), Rutenburg, Wohman & Seligman (1953) and Wachstein & Meisel (1955). The patterns obtained with other methods for enzymes of aerobic metabolism corresponded to those for SDHase (Dubowitz & Pearse, 1960*b*; Engel, 1962; Romanul, 1964). It has been claimed that the activity of the anaerobic metabolic enzyme phosphorylase is 'reciprocal' to that of enzymes of aerobic metabolism in skeletal muscle fibres of the rat and human (Dubowitz & Pearse, 1960*a*, *b*) and the cat (Jinnai, 1960). This concept has been supported by Engel (1962, 1965, 1970) and Suchenwirth & Bundschu (1970) in human muscles; Nishiyama (1966) in respiratory muscles of the rat and cat; Kugelberg & Edström (1968) in rat crural muscles; Moody & Cassens (1968) in the longissimus and trapezius muscles of the pig; and Jasmin, Bokdawala & Desrosiers (1971) in crural muscles of the hamster. However, fibres high in SDHase and moderate to high in phosphorylase activity have been demonstrated in rat crural muscles (Romanul, 1964), in rat femoral muscles and m. soleus of monkey and rat (Bocek & Beatty, 1966), in the thyroarytenoid and cricothyroid muscles of the rabbit (Hall-Craggs, 1968), in guinea-pig crural muscles (Edgerton & Simpson, 1969), in cat crural muscles (Prewitt & Salafsky, 1970), and in the gastrocnemius and soleus muscles of the mouse and the triceps brachii and rectus abdominis muscles of the pig and ox (Ashmore & Doerr, 1971). Using methods for SDHase, glycogen and myosin adenosine triphosphatase (myosin ATPase), Stein & Padykula (1962) established histochemical 'profiles' from transverse serial sections of fibres in rat crural muscles. They showed that some fibres are high in both myosin ATPase and SDHase activity. The existence of this type of fibre confounds the simple division of muscle fibres into two histochemical types as proposed by Engel (1962, 1965, 1970) for human muscles and applied to crural muscles of the guinea-pig by

Karpati & Engel (1967, 1968); their 'Type I' fibres have high activities for the enzymes of aerobic metabolism and show little phosphorylase or myosin ATPase activity, whereas their 'Type II' fibres have low activity for aerobic enzymes and high activity for phosphorylase and myosin ATPase. Edgerton & Simpson (1969) defined three basic fibre types in the crural muscles of the rat and guinea-pig; those with activities high for myosin ATPase, aerobic and anaerobic enzymes, those with activities high for myosin ATPase and anaerobic enzymes and low for aerobic enzymes, and those with activities low for myosin ATPase and anaerobic enzymes and showing 'intermediate' activity for aerobic enzymes. This 'intermediate' fibre frequently has, however, a higher SDHase activity than that of fibres high in myosin ATPase in muscles of the pig and ox (Ashmore & Doerr, 1971). Even this more complex classification does not seem to be generally applicable to all muscle fibres.

The present study was undertaken to resolve some of the apparently contradictory reports on the relationships between histochemical reactions in individual fibres. Patterns of fibre type profiles in one particular muscle have been established in mammals of different body size, using SDHase as an indicator of aerobic capacity, phosphorylase as an indicator of anaerobic capacity, and myosin ATPase as an indicator of the intrinsic speed of contraction of individual fibres. There has been no previous study comparing fibre type profiles in the same muscle of several different species. The diaphragm was chosen because this muscle has a similar function in all mammals and a comparable region, the costal diaphragm, can be sampled in each animal. The results have been briefly reported in a previous communication (Davies & Gunn, 1971).

MATERIAL AND METHODS

Preparation of material

Adult mice, rats, rabbits, cats, dogs and horses were killed by an overdose of anaesthetic. Adult sheep, pigs and cattle were killed by the usual abattoir procedures. The mice, rats and rabbits were laboratory strains reared in cages. The other animals used were from normal domestic and farm environments. The numbers and sex of the animals of each species are shown in Table 1. One sample of the costal diaphragm of each animal was removed within 45 minutes of death. Blocks of muscle, supported between blocks of liver tissue in the case of the smaller animals, were mounted on a cryostat chuck. A 5 mm thick cork sheet interposed between the chuck and the tissue prevented splitting of the tissue when chuck and tissue were rapidly frozen by plunging into dichlorodifluoromethane (Arcton 12, I.C.I.) cooled to its melting-point of -158 °C with liquid nitrogen. About ten adjacent serial transverse sections were cut 10 μm thick at -20 °C, mounted directly on to coverslips, and allowed to thaw and dry rapidly at room temperature.

Histochemical methods

Succinate dehydrogenase

Sections were incubated for 20 minutes at 37 °C in a medium composed of 10 ml of 0·2 M phosphate buffer at pH 7·6, 10 ml of 0·2 M sodium succinate, and 20 ml of nitro blue tetrazolium (1 mg/ml) (Nachlas *et al.* 1957). Gas bubbles frequently formed between the section and the coverslip; these were often eliminated by drying the section between washing and fixation in 4% formaldehyde.

Table 1. *Mean transverse-sectional areas of diaphragm muscle fibres*

Species	No. of animals			Transverse-sectional area (μm^2)		
	Male	Female	Total	Mean	S.D.	Significance*
Mouse (*Mus musculus*)	4	0	4	1110	160	$P < 0.05$
Rat (*Rattus rattus*)	3	1	4	1990	460	N.S.
Rabbit (*Oryctolagus cuniculus*)	3	0	3	3170	180	N.S.
Cat (*Felis catus*)	1	1	2	3590	1720	N.S.
Dog (*Canis familiaris*)	4	1	5	1690	150	N.S.
Sheep (*Ovis aries*)	0	3	3	1870	300	N.S.
Pig (*Sus scrofa*)	1	5	6	5680	1170	$P < 0.001$
Ox (*Bos taurus*)	1	2	3	3790	460	N.S.
Horse (*Equus caballus*)	3	1	4	2420	560	N.S.
Total	20	14	34	2690	1370	—

* Significance of difference of mean for each species and mean for all species, tested at the 5% significance level by the test quotient *t*.
N.S. = not significant.

Lipids

Sections were fixed in 4% formaldehyde for 10 minutes and stained with a freshly filtered, saturated solution of Sudan black B in 70% ethanol for 20 minutes. Excess dye was removed by a brief wash in 50% ethanol. Some sections were immersed in acetone for 30 minutes between fixation and staining.

Phosphorylase

Takeuchi's (1956) modification of the method of Takeuchi & Kuriaki (1955) was used. Sections were incubated for three hours at 37 °C in a medium consisting of 75 mg glucose-1-phosphoric acid, 15 mg adenosine-5′-monophosphoric acid, 3 mg glycogen, 22·5 ml distilled water, 15 ml of 0·1 M acetate buffer at pH 5·8, one international unit of protamine zinc insulin and 7·5 ml of absolute ethanol. They were subsequently washed, dried, fixed in absolute ethanol, dried, and stained with dilute Lugol's iodine for three minutes. Because the colour faded, iodine staining was repeated immediately before subsequent use of the section.

Myosin ATPase

The calcium–cobalt method of Padykula & Herman (1955) was modified to improve the buffering capacity of the medium. Sections were fixed for exactly two minutes in cacodylate buffered 4% formaldehyde at pH 7·0. Without fixation, the sections floated off the coverslip, and prolonged fixation affected the characteristics of the enzyme (Stein & Padykula, 1962; Guth & Samaha, 1969). Sections were incubated for 20 minutes at 37 °C in a freshly made medium consisting of 8 ml of 1·0 M tris-(hydroxymethyl)-aminomethane, 4 ml of 0·18 M calcium chloride, and 60 mg ATP disodium salt made up to 30 ml with distilled water, which was then adjusted to a pH of 9·5 with 0·1 N-HCl and made up to a final volume of 40 ml. The final concentration of ATP was therefore 2·4 mM. With two washes in distilled water between treatments, the sections were immersed in 2% cobalt chloride for three minutes and developed in dilute ammonium sulphide for one minute.

Cell outlines

Sections were fixed for 10 minutes in 4% formaldehyde, washed, and stained for 20 minutes in Ehrlich's haematoxylin.

Methods for establishing fibre profiles and transverse-sectional areas

Profiles of about 400 individual fibres in each section were established by first back-projecting a haematoxylin stained section on to a glass screen, enabling a tracing of the fibre outlines to be made on transparent paper. Each serial section was then projected in turn. The histochemical reaction of each fibre was indicated on the tracing; Figs. 3–7 illustrate the type of material used. To estimate the level of activity of enzymes that showed a continuous spectrum of activity between fibres, a simple division into 'high' and 'low' was made for each fibre, relative to the overall level of activity of fibres in each section. It was not considered possible to compare one sample with another, because of difficulties of standardization of the preparation and processing of the material. This is considered to be a source of variation in the quantitative data between samples, and precludes the possibility of a comparison between species based on overall enzyme activity. Fibre transverse-sectional areas were estimated by counting the number of fibres projected within an area of known magnification on the screen.

RESULTS

Qualitative histochemistry

Succinate dehydrogenase

Diformazan deposition occurred as blue dots or irregular areas that appeared to form a network around the myofibrils (Figs. 13, 15, 17, 19). In fibres with a high level of activity, diformazan deposition was highest in the subsarcolemmal region. Diformazan was deposited in larger aggregations in the smaller animals. It was frequently observed that fibres shown in serial section to be myosin ATPase-low had a pattern of intense blue, punctate, clearly defined dots, moderately dense and evenly distributed, whereas the colour of the reaction in ATPase-high fibres was purplish, especially in freshly stained sections. This variation in reaction was not sufficiently consistent to use in identifying fibre types.

Lipids

The density of staining with Sudan black B, with or without previous treatment of the section with acetone, always corresponded to SDHase activity (Figs. 5, 6, 7). SDHase activity was therefore chosen to determine histochemical profiles.

Phosphorylase

Fibres varied in reaction from an intense blue network to a paler blue, to a diffuse pink, to fibres coloured only by the iodine. In all species except the mouse, fibres high in both phosphorylase and SDHase activity were seen (Figs. 4, 6, 17, 18).

Table 2. *Percentage of histochemical fibre types in the diaphragm of nine mammalian species*

Species	No. of fibres counted	Percentage of fibre types* Ah Sh Ph	Ah Sh Pl	Ah Sl Ph	Ah Sl Pl	Al Sh Ph	Al Sh Pl	Al Sl Ph	Al Sl Pl	Sh	Ph	Ah	Contraction times† ms ± S.D.
Mouse	1436	—	93	—	—	—	7	—	—	100	—	93	—
Rat	1337	7	25	27	2	1	38	—	—	71	35	61	18 ± 1
Rabbit	1605	21	—	36	—	—	43	—	—	64	57	57	32 ± 4
Cat	1612	16	—	45	—	—	39	—	—	55	61	61	39 ± 2
Dog	3573	64	—	—	—	—	36	—	—	100	64	64	65 ± 5
Sheep	2938	43	—	—	—	43	14	—	—	100	86	43	—
Pig	2548	17	3	32	3	4	41	—	—	65	53	55	—
Ox	1879	24	—	—	—	58	18	—	—	100	82	24	—
Horse	2559	21	—	—	—	77	2	—	—	100	98	21	—

* Key: Ah, Sh, Ph: high activity of myosin ATPase, SDHase or phosphorylase respectively. Al, Sl, Pl: low enzyme activity.

† Reference for contraction times: Sant'Ambrogio & Saibene (1970).

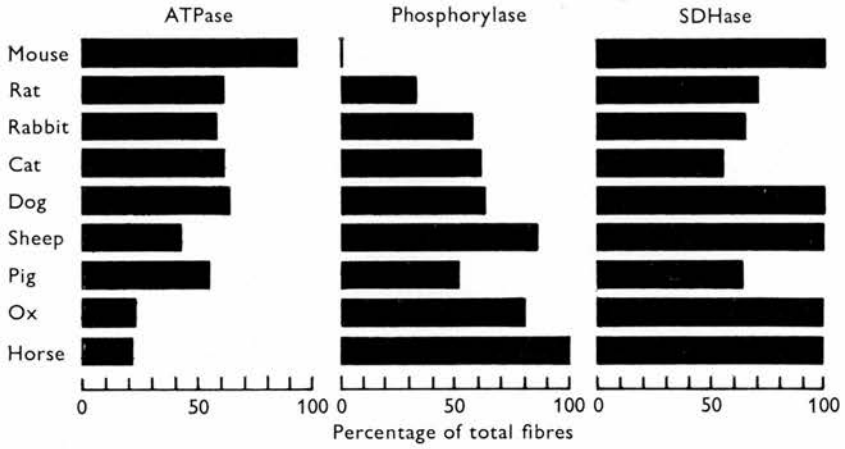


Fig. 1. Histogram showing the number of fibres of each species with high activity of myosin ATPase, phosphorylase and SDHase, as a percentage of total fibres sampled.

Myosin ATPase

Myosin ATPase-high fibres showed a dense brown reaction in which a brown network could usually be seen. These were always distinct from myosin ATPase-low fibres in which only the brown network was seen. Blood vessels were also stained; the reaction of capillary endothelia varied between species (Figs. 3, 9–12). Myosin ATPase-low fibres frequently had an activity of SDHase equal to or greater than adjacent myosin ATPase-high fibres (Figs. 3, 6, 13, 14, 15, 16, 19, 20); it was not therefore possible to grade the activity of SDHase from the myosin ATPase reaction.

Quantitative histochemistry

The data obtained on histochemical profiles of muscle fibres in the diaphragm are shown in Table 2 and Fig. 1. The proportion of myosin ATPase-high fibres decreases in the larger animals (Figs. 9–12). All myosin ATPase-low fibres are high in SDHase activity. A proportion of myosin ATPase-high fibres were classified as SDHase-low only in the rat, rabbit, cat and pig; hence the proportion of SDHase-high fibres is not dependent on body size. All fibres in the diaphragm of the dog (Fig. 8), for instance, are high in SDHase activity. The proportion of fibres high in phosphorylase activity increases in the larger animals. With the exception of all fibres in the mouse and a proportion of fibres in the rat and pig, all myosin ATPase-high fibres are high in phosphorylase activity. In the larger animals some myosin ATPase-low fibres are phosphorylase high. All species except the mouse have a significant proportion of fibres high in both phosphorylase and SDHase.

Mean transverse-sectional areas of diaphragm muscle fibres are shown in Table 1. Only two species are significantly different ($P < 0{\cdot}05$) from the mean of all the species; the mouse has small fibres and the pig large fibres relative to the other species.

DISCUSSION

Sources of variation

All samples were taken from the costal diaphragm of adult animals, which were of mixed sex and from different environments. There is no published work on the difference in the proportion of histochemical fibre types between sexes. Following artificial exercise in rats (Edgerton, Gerchman & Carrow, 1969) and guinea-pigs (Barnard, Edgerton & Peter, 1970), an increase was observed in the proportion of crural muscle fibres with high activity of enzymes of aerobic metabolism. There was, however, no significant difference in the proportion of myosin ATPase-high fibres. The effect of exercise on fibre types in the diaphragm has not been studied. The mice, rats and rabbits sampled in the present study had had very little exercise and the other species an unknown amount. With present evidence, it is possible that SDHase-low fibres in the diaphragm of the rat, rabbit, cat and pig are present because of lack of normal exercise. Since adult animals were used, the decrease reported in the proportion of myosin ATPase-high fibres in the developing soleus muscle of the guinea-pig, rat and cat (Karpati & Engel, 1967) and pectineus muscle of the dog (Cardinet *et al.* 1969) does not influence the results of this study. The variation in the proportion of myosin ATPase-high fibres between animals within a species is high, but does not obscure the overall effect of body size. We have observed large differences, which are not related to body size, in the proportion of myosin ATPase-high fibres in the diaphragm and limb muscles between breeds of dogs and horses (unpublished observations). The vertebral, sternal and costal regions of the rat diaphragm have been shown to vary in their lipase and SDHase activity (George & Susheela, 1961), respiratory quotient (Susheela & George, 1963) and butyrate oxidation (Susheela & George, 1964). However, Günther (1952, 1953) observed no differences in the proportion of fibres with 'Fibrillenstruktur' and 'Felderstruktur' in these regions of the diaphragm of the rat, mouse and dog, and Nishiyama (1966) stated that there was no variation

between regions of the cat diaphragm in the proportion of fibre types demonstrated by histochemical methods for oxidative enzymes and phosphorylase.

The number of species, their range of body size, and the number of animals of each species used in the present study indicate a consistent pattern of fibre type distribution, but more detailed studies are needed to establish differences between breeds and the manner in which these differences develop.

Transverse-sectional areas of muscle fibres

The diaphragm samples used in our study were all removed before rigor and were therefore contracted. Freezing and section cutting were not considered to alter fibre size appreciably. Transverse-sectional areas were estimated only in regions in which fibres were cut transversely and where the amount of endomysium and perimysium was minimal. We therefore consider that the estimates of transverse-sectional area are comparable between samples and species.

The relation of body size to the transverse-sectional area of fibres in an equivalent muscle of different species has not been studied extensively. Joubert (1956) found that fibre diameters in the gastrocnemius muscle of two sheep, two rabbits, one pig and one ox were not related to body size. George & Naik (1959) studied the pectoralis muscle of 25 species of birds of varying body weight. The mean fibre diameter increased with increasing weight of the muscle, at a rate dependent on the mode of flight. Julian & Cardinet (1961) found that the mean transverse-sectional area of fibres of m. biceps brachii of a wide range of breeds of dogs (1·4 kg to 56 kg) was larger in heavier dogs. The dogs used in the present study were in the middle of this weight range. Gauthier & Padykula (1966) reported the results of fibre measurements in the diaphragm of 13 mammalian species, and claimed that there was a direct relationship between fibre diameter and body size. They gave no data for the horse and dog, which we found to have small fibres relative to several smaller species.

Our results suggest that there are large sources of variation in fibre transverse-sectional area apart from body size. The diaphragm of the mouse has a purely aerobic metabolism; small fibres would facilitate a high rate of oxygen diffusion. On this basis, Hill (1956) predicted that the diameter of muscle fibres should vary as the square root of linear body size. However, the fibres in the diaphragms of the species which we have studied do not fit this hypothesis.

Significance of the histochemical reactions used

Succinate dehydrogenase

Patterns of mitochondria, demonstrated by classical methods (Nachlas *et al.* 1957; Scarpelli & Pearse, 1958; Novikoff, Shin & Drucker, 1961) or by electron microscopy in skeletal muscle (Padykula & Gauthier, 1963; Ogata, 1964; Pieper, Feustel & Hubner, 1969) and kidney (Novikoff *et al.* 1961), have in each case been shown to follow the diformazan deposition caused by SDHase activity. Brooke & Engel (1966) provided evidence that nitro BT is selectively adsorbed on to mitochondria and sarcoplasmic reticulum of striated muscle fibres. Since, however, SDHase is believed to be entirely intramitochondrial (Roodyn, 1967), this should enhance the histochemical localization of this enzyme. A limited extent of diformazan deposition away from

sites of SDHase activity, such as lipid droplets (Hitzeman, 1963), should have little effect on the comparison between individual fibres, but the report of a heterogeneous all-or-none deposition in individual mitochondria (Seligman *et al.* 1967) could have more serious implications. It is possible that the SDHase activity of mitochondria from different fibres may vary (Blanchaer, 1964), but the density of diformazan deposited histochemically in a particular fibre after incubation for as long as 20 minutes should depend primarily on mitochondrial density, rather than on the actual level of SDHase activity.

Paul & Sperling (1952) demonstrated a direct relationship between estimates of mitochondrial density, determined by phase microscopy of blendorized tissue, and the oxidative capacity of a variety of muscles from different species. This relationship is supported by observations on the effect of severe exercise on limb muscles, which can produce a twofold increase in the capacity of muscle to oxidize pyruvate (Holloszy, 1967), accompanied by a concomitant increase in mitochondrial density as seen electron microscopically (Gollnick & King, 1969). Similar findings were reported by Kraus, Kirsten & Wolff (1969).

The assumption that the histochemical SDHase reaction indicates the capacity of an individual fibre for aerobic metabolism appears reasonable, although it lacks direct proof.

Sudan black B

Padykula & Gauthier (1963) showed that this stain coloured both triglyceride droplets and phospholipid in muscle fibres of the rat diaphragm. Previous treatment with acetone removed triglyceride droplets but not phospholipid. They showed that the intracellular localization of the stain corresponded to the fat droplets and mitochondria seen electron microscopically. Since acetone extraction of our sections did not affect the density of the staining of an individual fibre relative to others surrounding it, triglyceride droplets appear to be associated with mitochondria in all fibres. The use of Sudan black B as a mitochondrial marker to estimate the dependence of a fibre on aerobic metabolism is as valid as the use of SDHase.

Phosphorylase

Takeuchi & Kuriaki (1955) showed that their method is specific for the enzyme catalysing the successive phosphorylation of the terminal glucose units of the glycogen chain, with the production of glucose-1-phosphate. The method uses the reversibility of this reaction to synthesize a polyglucose, staining blue with iodine, that is distinct from native glycogen, both by iodine staining and by electron microscopic appearance (Takeuchi & Sasaki, 1968). Differences in the colour of iodine staining have been attributed to the progressive increases in chain length during synthesis of the glucose polymer, blue indicating chains of over 30 glucose units, and red indicating chains of 7–13 glucose units (Swanson, 1948). Iodine colours have been used in this study to indicate different levels of phosphorylase activity in individual fibres.

It is accepted that glycogen is the major store of energy for muscular contraction in the absence of oxygen, and that phosphorylation is the first step in glycolysis. The

phosphorylase activity of an individual fibre is therefore a measure of the rate at which it can derive energy for contraction anaerobically.

Myosin ATPase

Padykula & Herman (1955) and Padykula & Gauthier (1963) provided evidence that their histochemical technique was specific for myosin ATPase. This was given strong support by the work of Guth & Samaha (1969), who compared the effects of pre-incubation at pH values of 10·4 and 4·35 on the ATPase activity of both actomyosin extracted from fast and slow muscles of the cat, and individual fibres of these muscles examined histochemically. Their study also provided evidence that fibres shown histochemically to be ATPase-high are fast contracting, and that ATPase-low fibres are slow contracting, a concept supported by work showing that the activity of myosin ATPase is directly proportional to the intrinsic speed of shortening of normal muscles of widely varying speeds of contraction (Bárány, 1967), and of muscles in which the speed of contraction has been altered by cross-innervation (Bárány & Close, 1971). The distinct difference between the histochemical reactions of fast and slow contracting fibres is possibly related to the molecular difference between the myosin of fast and slow muscles demonstrated by Samaha, Guth & Albers (1970).

This evidence appears to justify the designation of ATPase-high mammalian extrafusal fibres as fast-twitch, and ATPase-low fibres as slow-twitch fibres.

Classification of fibre types

The interpretation of histochemical fibre types should relate the reactions directly to the physiological and metabolic characteristics of each fibre. The evidence given above suggests that the profile obtained by determining the SDHase, phosphorylase and myosin ATPase reactions will classify an individual fibre by its capacity for aerobic and anaerobic metabolism, and by its intrinsic speed of contraction. Fig. 2 is an attempt to classify fibre types in the diaphragm by these criteria. Where the incidence of a fibre is less than 5 %, it probably plays an insignificant part in the function of the diaphragm, and it is appreciated that a fibre classified as 'anaerobic' or 'aerobic' will usually have a low level of the other type of metabolism. For both fast- and slow-twitch fibres, there are three theoretical possibilities for their metabolism; aerobic, combined aerobic and anaerobic, and anaerobic. Only five of these six possibilities occur in significant proportions. The following trends are observed:

(i) Slow-twitch fibres use aerobic metabolism to a greater extent than fast-twitch fibres, and consequently do not include an 'anaerobic' type.

(ii) The diaphragm of the smaller animals uses a predominantly aerobic metabolism. Although a capacity for aerobic metabolism is maintained in the larger animals, there is also an increasing capacity for anaerobic metabolism.

Our studies demonstrate that fibres may have a high activity of enzymes for both aerobic and anaerobic metabolism. They do not indicate what the absolute levels of the activities of these enzymes might be, but suggest that the relationship between aerobic and anaerobic metabolism in muscle fibres is not necessarily the simple 'reciprocal' one suggested by Dubowitz & Pearse (1960*a*, *b*). This contention is supported by Gillespie, Simpson & Edgerton (1970), who found greater stores of glycogen in the 'red' region of m. vastus lateralis of the guinea-pig, composed of 77 % SDHase high

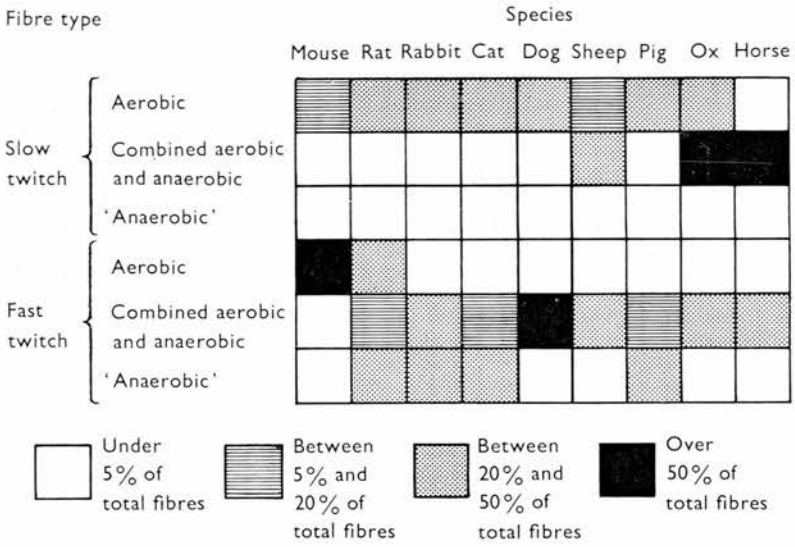


Fig. 2. Incidence of fibre types in the diaphragm.

fibres, than in the 'white' region, composed of 29% SDHase high fibres. Edgerton & Simpson (1969) reviewed the various classifications that have been used since 1962 for histochemical fibre types in muscle. They favoured the descriptive terms 'red', 'intermediate' and 'white' in preference to letters or numbers. Fibres low in myosin ATPase activity were described as 'intermediate' in SDHase activity by Stein & Padykula (1962), Edgerton & Simpson (1969) and Jasmin *et al.* (1971) in their studies of crural muscles of rat, guinea-pig and rat, and hamster respectively. Our results, and those of Ashmore & Doerr (1971) for limb muscles of the pig and ox, and Burke *et al.* (1971) for the cat gastrocnemius, show that this type of fibre frequently has SDHase activity equal to or greater than surrounding myosin ATPase high fibres. The term 'intermediate' has, therefore, no general significance.

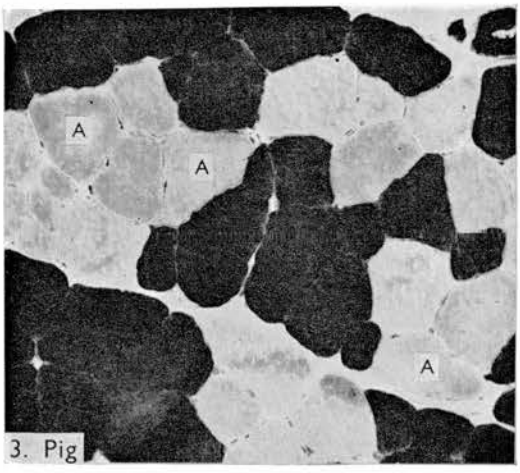
Muscle fibre types in the diaphragm

Bullard (1919) stained sections of the cat diaphragm with Sudan III and described equal numbers of 'dark' fibres with many lipid droplets and 'light' fibres of greater diameter and fewer lipid droplets, together with a small number of intermediate fibres. Günther (1952) also recognized the heterogeneity of muscle fibres in the diaphragm. By studying susa-fixed, paraffin embedded sections stained with Heidenhain's

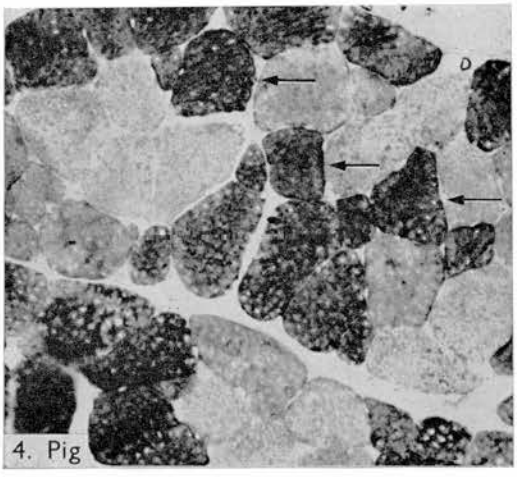
---

Figs. 3–7. Transverse serial sections of the pig diaphragm stained for myosin ATPase (Fig. 3), phosphorylase (Fig. 4), phospholipid and triglycerides (Sudan black B) (Fig. 5), SDHase (Fig. 6), and phospholipid (Sudan black B) after extraction of triglycerides with acetone (Fig. 7). Arrows indicate fibres high in both phosphorylase and SDHase activity. Fibres marked A have low myosin ATPase activity, but their SDHase activity and Sudanophilia are higher than any myosin ATPase high fibres.

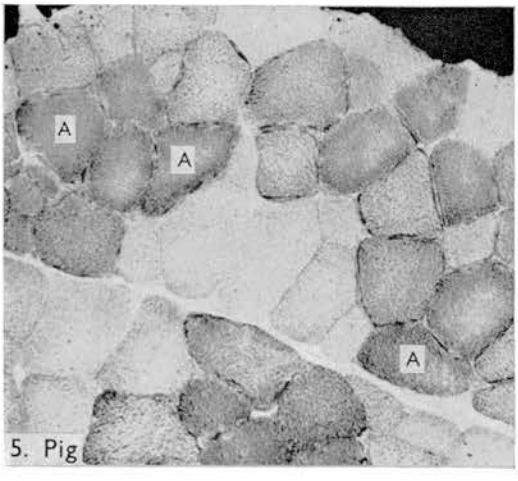
Fig. 8. Transverse section of diaphragm of dog: SDHase.



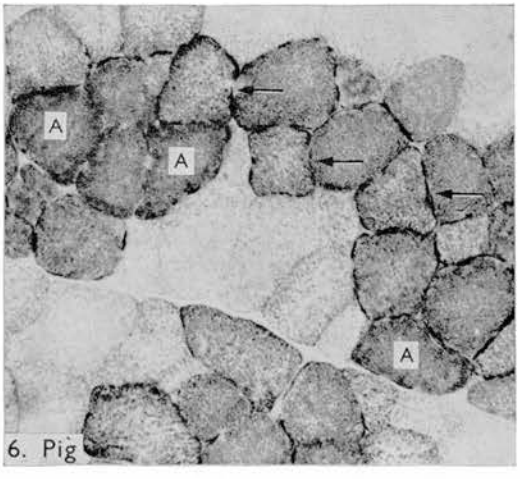
3. Pig



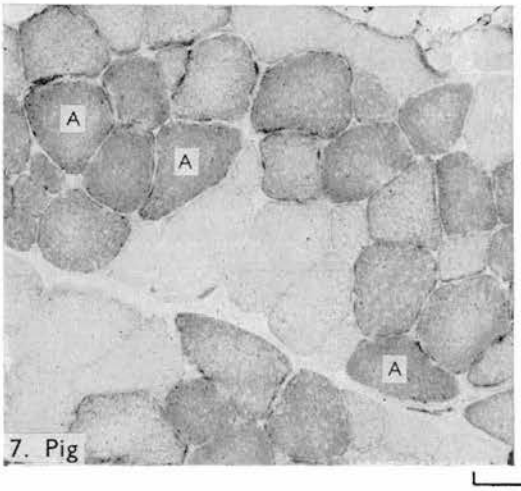
4. Pig



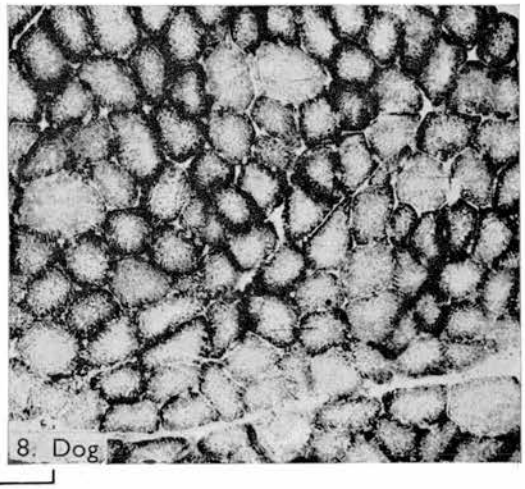
5. Pig



6. Pig

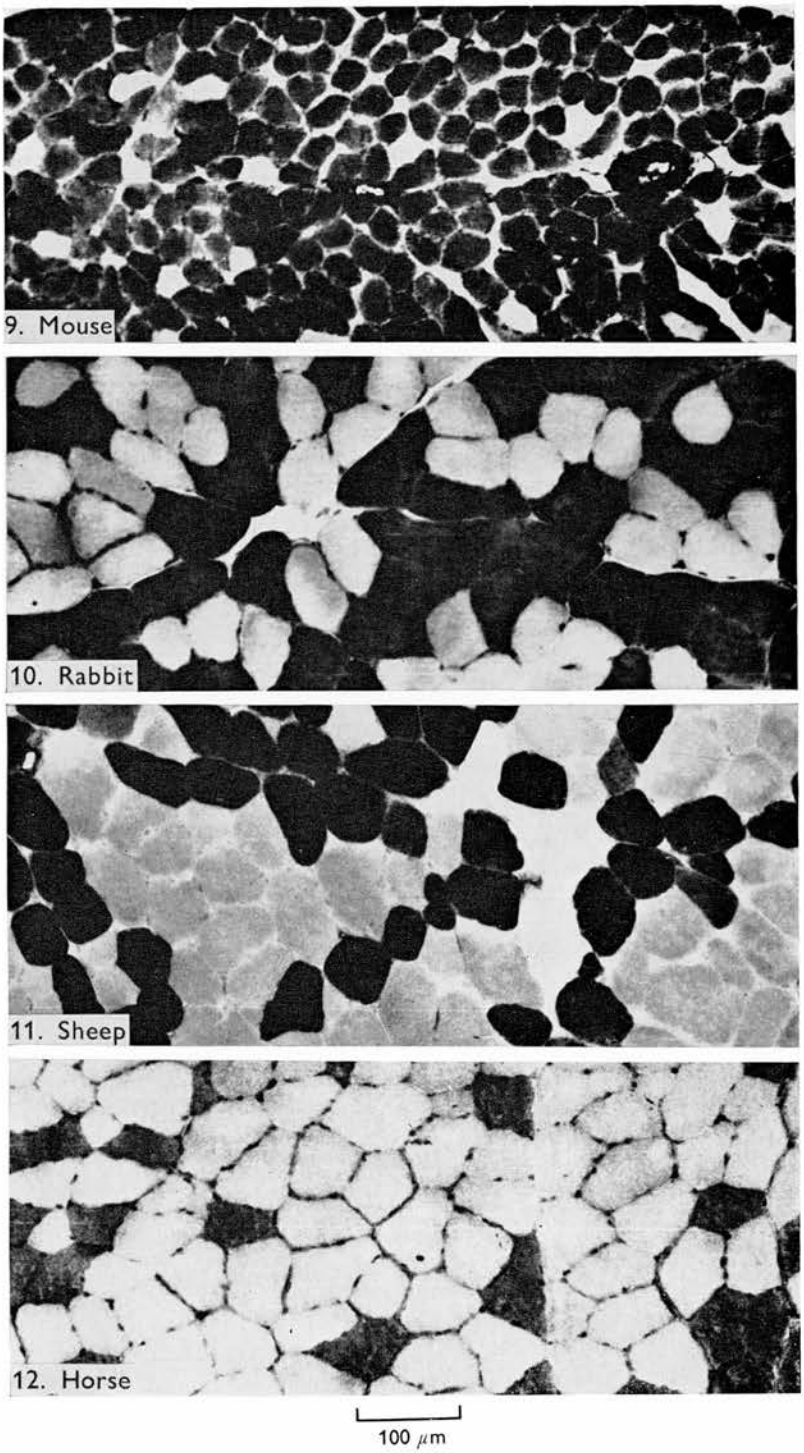


7. Pig



8. Dog

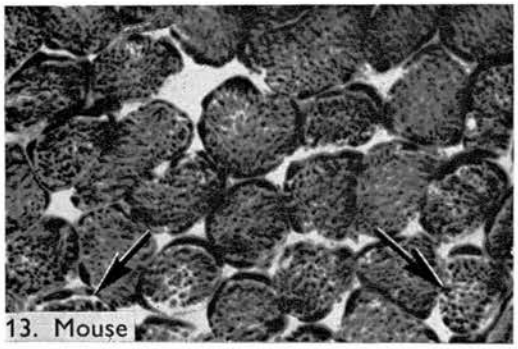
100 μm

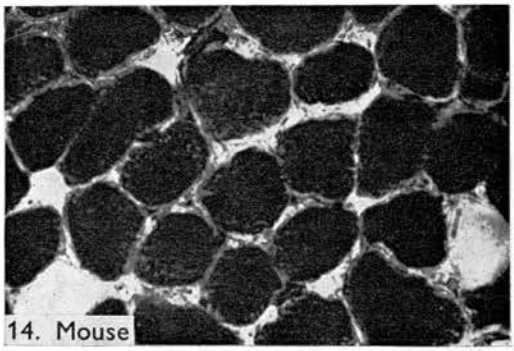


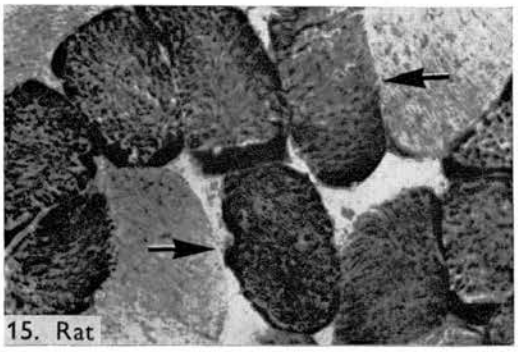
Figs. 9–12. Transverse sections of the diaphragm of the mouse (Fig. 9), rabbit (Fig. 10), sheep (Fig. 11), and horse (Fig. 12); myosin ATPase.

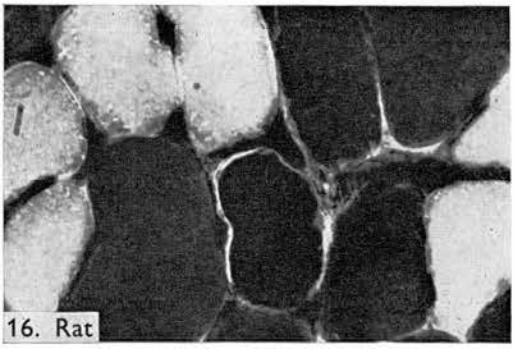
iron-alum haematoxylin, he reported the presence of two distinct populations of fibres in the diaphragm of the rat, mouse, rabbit and hedgehog, and later in the dog and human (Günther, 1953), corresponding in myofibrillar arrangement to the 'Fibrillenstruktur' and 'Felderstruktur' fibres described by Krüger (1952). This morphological difference was considered to be correlated with 'phasic' and 'tonic' contraction respectively; in the rat diaphragm 42% of fibres were classified as 'Felderstruktur', a proportion close to the 39% of ATPase-low fibres that we have observed. However, Günther (1953) found only 5% of the fibres of the diaphragm of the dog to be 'Felderstruktur', compared with our 36% myosin ATPase-low fibres in this species. In an electron microscopic study of the rat diaphragm, Bubenzer (1966) described two fibre types; thin, mitochondria-rich fibres were 'Felderstruktur', and thick fibres with fewer mitochondria, less glycogen and no lipid droplets were 'Fibrillenstruktur'. Muscle fibres in the diaphragm of the mouse, rat, guinea-pig and rabbit were found to vary in their phosphorylase activity by Takeuchi & Kuriaki (1955). Histochemical methods for enzymes of oxidative metabolism in the diaphragm of several mammals (Ogata, 1958) and in the diaphragm of the rat (George & Susheela, 1961; Padykula & Gauthier, 1963) showed a large variation in enzymic activity of individual fibres. Nishiyama (1966) distinguished three fibre types in the rat and cat diaphragm and concluded that phosphorylase was reciprocal to the activity of oxidative enzymes. However, we have observed a significant proportion of fibres in these muscles that contain high levels of both phosphorylase and SDHase activity (Figs. 17, 18).

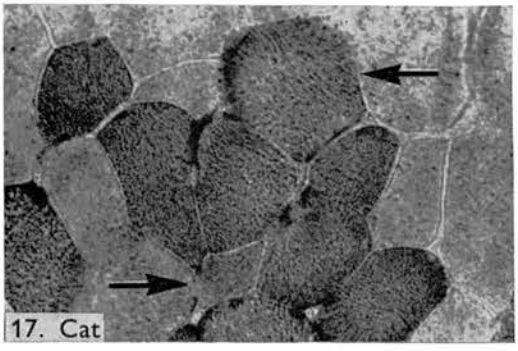
Padykula & Gauthier (1963) used the myosin ATPase reaction on the rat diaphragm but did not report a variation between fibres. In this muscle, they distinguished by electron microscopy 'red', 'intermediate' and 'white' fibres based on mitochondrial density, fibre diameter, width of Z line, and end-plate morphology (Padykula & Gauthier, 1963, 1970; Gauthier & Padykula, 1966). Gauthier & Padykula (1966) compared fibre types in the diaphragm of the rat, bat, shrew and ox with the electron microscope. They also studied the costal diaphragm of 36 mammalian species using Sudan black B staining of material either fresh frozen, or fixed in formalin or osmium tetroxide, and found that the level of Sudanophilia decreased with increasing body size. The diaphragm of small animals (species of mouse, bat and shrew) was stated to be composed of 'homogeneous red fibres', that of animals intermediate in size (including rat, cat, rabbit, dog and sheep) of 'mixtures of fibre types', and that of the pig and ox of 'homogeneous white fibres'. Our findings differ in that the diaphragms of the dog and sheep are composed more or less uniformly of fibres of high SDHase activity and Sudanophilia (Fig. 8). The diaphragm of the pig is clearly heterogeneous when stained with Sudan black B (Figs. 5, 7). Although they do not show the dense aggregations seen in fibres of smaller animals (Figs. 13, 15), the fibres of the ox and horse diaphragm have relatively high Sudanophilia and SDHase activity (Fig. 19).

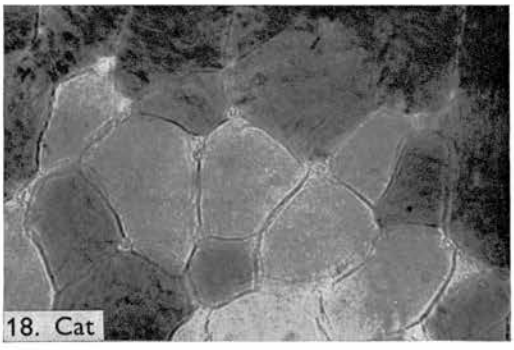

13. Mouse

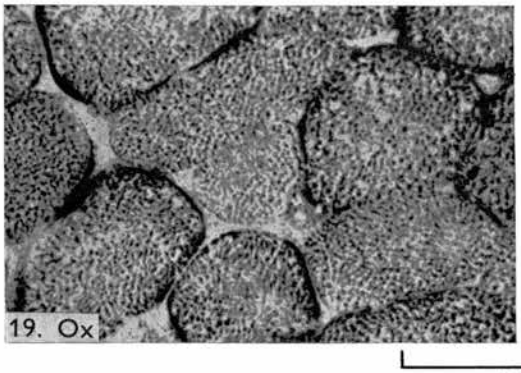

14. Mouse

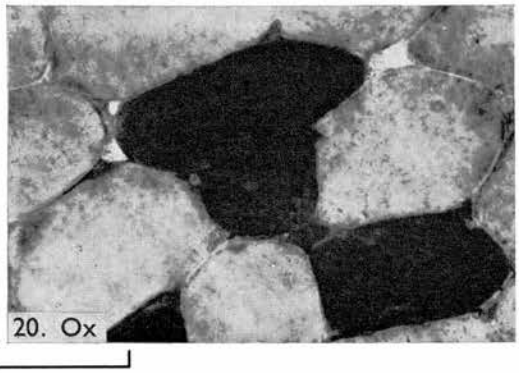

15. Rat


16. Rat


17. Cat


18. Cat


19. Ox


20. Ox

100 μm

Table 3. *Fibre types in muscles of known contraction times*

		Percentage of fibre type*											
		Ah				Al							
		Sh		Sl		Sh		Sl					Contraction time†
Species	Muscle	Ph	Pl	Ph	Pl	Ph	Pl	Ph	Pl	Sh	Ph	Ah	(ms)
Rabbit	Thyroarytenoid	100	—	—	—	—	—	—	—	100	100	100	6
Rat	E.D.L.*	63	—	33	—	4	—	—	—	67	100	96	12
Cat	F.D.L.*	33	2	56	—	—	9	—	—	44	89	91	27
Rabbit	Cricothyroid	1	77	—	—	4	18	—	—	100	5	78	27
Rat	Soleus	14	1	—	—	1	84	—	—	100	15	15	36
Cat	Soleus	—	—	—	—	—	100	—	—	100	—	—	70

* Key: Ah, Sh, Ph: high activity of myosin ATPase, SDHase or phosphorylase respectively. Al, Sl, Pl: low enzyme activity. F.D.L.: m. flexor digitorum longus. E.D.L.: m. extensor digitorum longus.

† References for contraction times: Cat (Buller, Eccles & Eccles, 1960); rat (Close, 1964); rabbit (Hall-Craggs, 1968).

Comparative physiology

The presence of fast- and slow-twitch fibres in the diaphragm has been indicated by our histochemical demonstration of myosin ATPase-high and low fibres in all animals studied. Physiological evidence for this has been provided by studies on the action potentials in single diaphragmatic muscle fibres of the rabbit (Sant'Ambrogio, Decandia & Gantchev, 1969), and on the firing patterns of single phrenic motoneurons of the cat (Nail, Sterling & Widdicombe, 1969), which demonstrated the presence of two types of motor unit, differing in their threshold for stimulation and in firing frequency. For motor units of the cat gastrocnemius muscle these properties have been shown to be related to contraction times (Burke, 1968).

We have studied fibre types in muscles of known contraction times. The results are shown in Table 3, and confirm the postulation of Edgerton & Simpson (1969) that the proportion of myosin ATPase-high fibres in these muscles bears a reciprocal relationship to the contraction time. The histochemical findings of Barnard, Edgerton, Furukawa & Peter (1971) in guinea-pig hind limb muscles, and of Cardinet,

Figs. 13, 14. Transverse serial sections of the diaphragm of the mouse, stained for SDHase (Fig. 13), and myosin ATPase (Fig. 14). Arrows indicate myosin ATPase-low fibres with similar SDHase (Fig. 13), and myosin ATPase (Fig. 14). Arrows indicate myosin ATPase-low fibres with similar SDHase activity to myosin ATPase-high fibres.

Figs. 15, 16. Transverse serial sections of the diaphragm of the rat, stained for SDHase (Fig. 15) and myosin ATPase (Fig. 16). Arrows indicate fibres high in both SDHase and myosin ATPase activity.

Figs. 17, 18. Transverse serial sections of the diaphragm of the cat, stained for SDHase (Fig. 17) and phosphorylase (Fig. 18). Arrows indicate fibres high in both SDHase and phosphorylase activity.

Figs. 19, 20. Transverse serial sections of the diaphragm of the ox, stained for SDHase (Fig. 19) and myosin ATPase (Fig. 20). The myosin ATPase-low fibres have similar, or higher, SDHase activity compared with the myosin ATPase-high fibres.

Tunell & Fedde (1971) in m. pectineus of dogs, support this hypothesis. Table 2 shows the contraction times determined for the diaphragm of the rat, rabbit, cat and dog by Sant'Ambrogio & Saibene (1970). Although their results suggest that contraction time is related to body size, our data show little variation in the proportion of myosin ATPase-high fibres between these species. A histochemical study of the actual diaphragms for which contraction times have been determined will be necessary to resolve this inconsistency.

The proportion of myosin ATPase-high fibres in the semitendinosus muscle also appears to be inversely proportional to body size (Davies & Gunn, 1971), although the effect is not as marked as in the diaphragm. This property of m. semitendinosus would be expected by the requirements of dimensional theory (Hill, 1950), for which the limb muscles of larger animals must have lower intrinsic speeds of contraction.

Comparative metabolism

Crosfill & Widdicombe (1961) showed that in mice and rats the frequency of breathing could vary widely for constant alveolar ventilation with little increase in work, and that the rate of work of breathing per gram of body weight was high, compared with that in the larger guinea-pigs, rabbits, monkeys, cats and dogs. Since myosin ATPase activity is probably the rate-limiting step in the conversion of chemical to mechanical energy in muscle (Mommaerts, 1970), their results are consistent with the proportions of myosin ATPase-high fibres that we have seen in the diaphragm of the mouse, rat, rabbit, cat and dog. It is probable, then, that the diaphragms of the larger animals used in our study will have even lower rates of energy conversion than the larger animals studied by Crosfill & Widdicombe (1961).

Kunkel, Spalding, de Franciscis & Futrell (1956) showed that the cytochrome oxidase activity per gram of gracilis muscle of the rat, sheep, pig and ox varied as the $-0{\cdot}24$ power of body weight, and therefore directly with the metabolic rate (Kleiber, 1947). Although the respiration rate did not vary with body weight in other muscles, tissue slices from the diaphragm of a series of mice and rats respired at a rate proportional to the $-0{\cdot}15$ power of body weight (Bertalanffy & Estwick, 1953). While it is expected, therefore, that the capacity for oxidative metabolism of the diaphragm in our series of animals will also be less with increasing body size, fibres of the diaphragm of the larger animals appear histochemically to be dependent on aerobic metabolism. They do, however, have a capacity for anaerobic metabolism, presumably for brief periods of greater effort.

SUMMARY

Histochemical profiles of individual muscle fibres from the diaphragm of the mouse, rat, rabbit, cat, dog, sheep, pig, ox and horse were classified according to their reaction to methods demonstrating myosin adenosine triphosphatase, succinate dehydrogenase and phosphorylase. Other evidence indicates that the myosin adenosine triphosphatase reaction differentiates between fast-twitch and slow-twitch muscle fibres and that the succinate dehydrogenase and phosphorylase reactions demonstrate the capacity for aerobic and anaerobic metabolism respectively. Fast-twitch fibres, which may use either an aerobic metabolism, an anaerobic metabolism or a com-

bined aerobic and anaerobic metabolism, and slow-twitch fibres, which may use either an aerobic metabolism or a combined aerobic and anaerobic metabolism, have been recognized in the diaphragm.

The diaphragm of smaller animals has a high proportion of fast-twitch fibres and a predominantly aerobic metabolism. The diaphragm of larger animals has a majority of slow-twitch fibres and a capacity for combined aerobic and anaerobic metabolism. Only the mouse and pig have mean fibre transverse-sectional areas significantly different from the mean of all the species studied.

We are indebted to Professor A. R. Muir for assistance in all stages of this study. One of us (A. S. D.) was supported by a grant from the Meat and Livestock Commission.

REFERENCES

ASHMORE, C. R. & DOERR, L. (1971). Comparative aspects of muscle fiber types in different species. *Experimental Neurology* **31**, 408–418.

BÁRÁNY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *Journal of General Physiology* **50**, 197–218.

BÁRÁNY, M. & CLOSE, R. (1971). The transformation of myosin in cross-innervated rat muscles. *Journal of Physiology, London* **213**, 455–474.

BARNARD, R. J., EDGERTON, V. R., FURUKAWA, T. & PETER, J. B. (1971). Histochemical, biochemical and contractile properties of red, white and intermediate fibers. *American Journal of Physiology* **220**, 410–414.

BARNARD, R. J., EDGERTON, V. R., PETER, J. B. (1970). Effect of exercise on skeletal muscle. I. Biochemical and histochemical properties. *Journal of applied Physiology* **28**, 762–766.

BERTALANFFY, L. VON & ESTWICK, R. R. (1953). Tissue respiration of musculature in relation to body size. *American Journal of Physiology* **173**, 58–60.

BLANCHAER, M. C. (1964). Respiration of mitochondria of red and white skeletal muscle. *American Journal of Physiology* **206**, 1015–1020.

BOCEK, R. M. & BEATTY, C. H. (1966). Glycogen synthetase and phosphorylase in red and white muscle of rat and rhesus monkey. *Journal of Histochemistry and Cytochemistry* **14**, 549–559.

BROOKE, M. H. & ENGEL, W. K. (1966). Nitro blue tetrazolium: selective binding within striated muscle fibers. *Neurology, Minneapolis* **16**, 799–806.

BUBENZER, H. J. (1966). Die dünnen und die dicken Muskelfasern des Zwerchfells der Ratte. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **69**, 520–550.

BULLARD, H. H. (1919). Histological as related to physiological and chemical differences in certain muscles of the cat. *Johns Hopkins Hospital Reports* **18**, 323–328.

BULLER, A. J., ECCLES, J. C. & ECCLES, R. M. (1960). Differentiation of fast and slow mucles in the cat hind limb. *Journal of Physiology, London* **150**, 399–416.

BURKE, R. E. (1968). Firing patterns of gastrocnemius motor units in the decerebrate cat. *Journal of Physiology, London* **196**, 631–654.

BURKE, R. E., LEVINE, D. N., ZAJAC, F. E., TSAIRIS, P. & ENGEL, W. K. (1971). Histochemical profiles of three physiologically defined types of motor units in cat gastrocnemius muscle. *Science* **174**, 709–712.

CARDINET, G. H., TUNELL, G. L. & FEDDE, M. R. (1971). A comparative study of contractile and histochemical properties in normal and hypotrophic muscle in the dog. *Anatomical Record* **169**, 288–289.

CARDINET, G. H., WALLACE, L. J., FEDDE, M. R., GUFFY, M. M. & BARDENS, J. W. (1969). Developmental myopathy in the canine with Type II muscle fiber hypotrophy. *Archives of Neurology* **21**, 620–630.

CLOSE, R. (1964). Dynamic properties of fast and slow skeletal muscles of the rat during development. *Journal of Physiology, London* **173**, 74–95.

CROSFILL, M. L. & WIDDICOMBE, J. G. (1961). Physical characteristics of the chest and lungs and the work of breathing in different mammalian species. *Journal of Physiology, London* **158**, 1–14.

DAVIES, A. S. & GUNN, H. M. (1971). A comparative histochemical study of the mammalian diaphragm and m. semitendinosus. *Journal of Anatomy* **110**, 137–139.

DUBOWITZ, V. & PEARSE, A. G. E. (1960*a*). Reciprocal relationship of phosphorylase and oxidative enzymes in skeletal muscle. *Nature, London* **185**, 701–702.

DUBOWITZ, V. & PEARSE, A. G. E. (1960*b*). A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. *Histochemie* **2**, 105–117.

EDGERTON, V. R., GERCHMAN, L. & CARROW, R. (1969). Histochemical changes in rat skeletal muscle after exercise. *Experimental Neurology* **24**, 110–123.

EDGERTON, V. R. & SIMPSON, D. R. (1969). The intermediate muscle fiber of rats and guinea pigs. *Journal of Histochemistry and Cytochemistry* **17**, 828–838.

ENGEL, W. K. (1962). The essentiality of histo- and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. *Neurology, Minneapolis* **12**, 778–794.

ENGEL, W. K. (1965). Diseases of the neuromuscular junction and muscle. In *Neurohistochemistry* (Ed. C. W. M. Adams). Amsterdam: Elsevier.

ENGEL, W. K. (1970). Selective and nonselective susceptibility of muscle fiber types: a new approach to human neuromuscular diseases. *Archives of Neurology* **22**, 97–117.

GAUTHIER, G. F. & PADYKULA, H. A. (1966). Cytochemical studies of fiber types in skeletal muscle. A comparative study of the mammalian diaphragm. *Journal of Cell Biology* **28**, 333–354.

GEORGE, J. C. & NAIK, R. M. (1959). Studies on the structure and physiology of the flight muscles of birds. 6. Variation in the diameter of the fibres of the pectoralis major and its relation to the muscle size and mode of flight. *Journal of Animal Morphology and Physiology* **6**, 90–94.

GEORGE, J. C. & SUSHEELA, A. K. (1961). A histophysiological study of the rat diaphragm. *Biological Bulletin* **121**, 471–480.

GILLESPIE, C. A., SIMPSON, D. R. & EDGERTON, V. R. (1970). High glycogen content of red as opposed to white skeletal muscle fibers of guinea pigs. *Journal of Histochemistry and Cytochemistry* **18**, 552–558.

GOLLNICK, P. D. & KING, D. W. (1969). Effect of exercise and training on mitochondria of rat skeletal muscle. *American Journal of Physiology* **216**, 1502–1509.

GÜNTHER, P. G. (1952). Die morphologischen Grundlagen der Bewegungs- und Haltleistung (Tetanus und Tonus) des Zwerchfells. *Acta anatomica* **14**, 54–64.

GÜNTHER, P. G. (1953). Das muskuläre Substrat der Bewegungs- und Haltleistung des menschlichen Zwerchfells. *Acta anatomica* **17**, 348–352.

GUTH, L. & SAMAHA, F. J. (1969). Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Experimental Neurology* **25**, 138–152.

HALL-CRAGGS, E. C. B. (1968). The contraction times and enzyme activity of two rabbit laryngeal muscles. *Journal of Anatomy* **102**, 241–255.

HILL, A. V. (1950). The dimensions of animals and their muscular dynamics. *Science Progress* **38**, 209–230.

HILL, A. V. (1956). The design of muscles. *British Medical Bulletin* **12**, 165–166.

HITZEMAN, J. W. (1963). Observations on the subcellular localisation of oxidative enzymes with nitro blue tetrazolium. *Journal of Histochemistry and Cytochemistry* **11**, 62–70.

HOLLOSZY, J. O. (1967). Biochemical adaptation in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *Journal of Biological Chemistry* **242**, 2278–2282.

JASMIN, G., BOKDAWALA, F. & DESROSIERS, M. (1971). Identification de la fibre intermédiaire dans le muscle squelettique. *L'Union médicale du Canada* **100**, 706–708.

JINNAI, D. (1960). Functional differentiation of skeletal muscles. *Acta medicinae Okayama* **14**, 159–169.

JOUBERT, D. M. (1956). An analysis of factors influencing post-natal growth and development of the muscle fibre. *Journal of Agricultural Science* **47**, 59–102.

JULIAN, L. M. & CARDINET, G. H. (1961). Fiber sizes of the biceps brachii muscle of dogs which differ greatly in body size. *Anatomical Record* **139**, 243.

KARPATI, G. & ENGEL, W. K. (1967). Neuronal trophic function: a new aspect demonstrated histochemically in developing soleus muscle. *Archives of Neurology* **17**, 542–545.

KARPATI, G. & ENGEL, W. K. (1968). Correlative histochemical study of skeletal muscle after supraspegmental denervation, peripheral nerve section and skeletal fixation. *Neurology, Minneapolis* **18**, 681–692.

KLEIBER, M. (1947). Body size and metabolic rate. *Physiological Reviews* **27**, 511–541.

KRAUS, H., KIRSTEN, R. & WOLFF, J. R. (1969). Die Wirkung von Schwimm- und Lauftrainung auf die celluläre Function und Struktur des Muskels. *Archiv für die gesamte Physiologie des Menschen und der Tiere* **308**, 57–79.

KRÜGER, P. (1952). *Tetanus und Tonus der quergestreiften Skelettmuskeln der Wirbeltiere und des Menschen*. Leipzig: Akademische Verlagsgesellschaft.

KUGELBERG, E. & EDSTRÖM, L. (1968). Differential histochemical effects of muscle contractions on phosphorylase and glycogen in various types of fibres; relation to fatigue. *Journal of Neurology, Neurosurgery and Psychiatry* **31**, 415–423.

KUNKEL, H. O., SPALDING, J. F., DE FRANCISCIS, G. & FUTRELL, M. F. (1956). Cytochrome oxidase activity and body weight in rats and three species of large animals. *American Journal of Physiology* **186**, 203–206.

MOMMAERTS, W. F. H. M. (1970). The role of the innervation of the functional differentiation of muscle. In *The Physiology and Biochemistry of Muscle as a Food* (Ed. E. J. Briskey, R. G. Cassens & B. B. Marsh) **2**, 53–66. Madison: University of Wisconsin Press.

MOODY, W. G. & CASSENS, R. G. (1968). Histochemical differentiation of red and white muscle fibers. *Journal of Animal Science* **27**, 961–968.

NACHLAS, M. M., TSOU, K., DE SOUZA, E., CHENG, C. & SELIGMAN, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of new p-nitrophenyl substituted ditetrazole. *Journal of Histochemistry and Cytochemistry* **5**, 420–436.

NAIL, B. S., STERLING, G. M. & WIDDICOMBE, J. G. (1969). Some properties of single phrenic motoneurones. *Journal of Physiology, London* **200**, 137*P*–138*P*.

NISHIYAMA, A. (1966). Histochemical studies on the red, white and intermediate muscle fibers of some skeletal muscles. III. Histochemical demonstration of oxidative enzymes, phosphorylase and glycogen in respiratory muscle fibers. *Acta medicinae Okayama* **20**, 137–146.

NOVIKOFF, A. B., SHIN, W. Y. & DRUCKER, J. (1961). Mitochondrial localisation of oxidative enzymes. Staining results with two tetrazolium salts. *Journal of Biophysical and Biochemical Cytology* **9**, 47–56.

OGATA, T. (1958). A histochemical study of the red and white muscle fibers. Part I. Activity of the succinoxidase system in muscle fibers. *Acta medicinae Okayama* **12**, 216–227.

OGATA, T. (1964). An electron microscopic study on the red, white and intermediate muscle fibers of the mouse. *Acta medicinae Okayama* **18**, 271–280.

PADYKULA, H. A. (1952). The localization of succinic dehydrogenase in tissue sections of the rat. *American Journal of Anatomy* **91**, 107–146.

PADYKULA, H. A. & GAUTHIER, G. F. (1963). Cytochemical studies of adenosine triphosphatases in skeletal muscle fibers. *Journal of Cell Biology* **18**, 87–107.

PADYKULA, H. A. & GAUTHIER, G. F. (1970). The ultrastructure of the neuromuscular junctions of mammalian red, white and intermediate skeletal muscle fibers. *Journal of Cell Biology* **46**, 27–41.

PADYKULA, H. A. & HERMAN, E. (1955). The specificity of the histochemical method for adenosine triphosphatase. *Journal of Histochemistry and Cytochemistry* **3**, 170–195.

PAUL, M. H. & SPERLING, E. (1952). Cyclophorase system. XXIII. Correlation of cyclophorase activity and mitochondrial density in striated muscle. *Proceedings of the Society for Experimental Biology and Medicine* **79**, 352–354.

PIEPER, K.-S., FEUSTEL, G. & HÜBNER, H.-J. (1969). Zur Localisation der Succinodehydrogenase in 'roten' (dünnen) Skelettmuskelfasern der weissen Ratte. *Acta histochemica* **33**, 171–178.

PREWITT, M. A. & SALAFSKY, B. (1970). Enzymic and histochemical changes in fast and slow muscles after cross-innervation. *American Journal of Physiology* **218**, 69–74.

RANVIER, L. (1873). Propriétés et structures différentes des muscles rouges et des muscles blancs chez les lapins et chez les raies. *Compte rendu de l'Académie des Sciences, Paris* **77**, 1030–1034.

ROMANUL, F. C. A. (1964). Enzymes in muscle: I. Histochemical studies of enzymes in individual muscle fibers. *Archives of Neurology* **11**, 355–368.

ROODYN, D. B. (1967). The mitochondrion. In *Enzyme Cytology* (Ed. D. B. Roodyn), pp. 103–180. London: Academic Press.

RUTENBERG, A. M., WOHMAN, M. & SELIGMAN, A. M. (1953). Comparative distribution of succinic dehydrogenase in six mammals and modification in the histochemical technic. *Journal of Histochemistry and Cytochemistry* **1**, 66–81.

SAMAHA, F. J., GUTH, L. & ALBERS, R. W. (1970). Differences between slow and fast muscle myosin. Adenosine triphosphatase activity and release of associated proteins by p-chloromercuriphenylsulfonate. *Journal of Biological Chemistry* **245**, 219–224.

SANT'AMBROGIO, G., DECANDIA, M. & GANTCHEV, N. G. (1969). The composite structure of the diaphragm of the rabbit. *Archivio di Fisiologia* **67**, 27–39.

SANT'AMBROGIO, G. & SAIBENE, F. (1970). Contractile properties of the diaphragm in some mammals. *Respiration Physiology* **10**, 349–357.

SCARPELLI, D. G. & PEARSE, A. G. E. (1958). Cytochemical localisation of succinic dehydrogenase in mitochondria. *Anatomical Record* **132**, 133–152.

SELIGMAN, A. M., UENO, H., MORIZONO, Y., WASSERKRUG, H., KATZOFF, L. & HANKER, J. (1967). Electron microscopic demonstration of dehydrogenase activity with a new osmiophilic ditetrazolium salt (TC-NBT). *Journal of Histochemistry and Cytochemistry* **15**, 1–13.

STEIN, J. M. & PADYKULA, H. A. (1962). Histochemical classification of individual skeletal muscle fibers of the rat. *American Journal of Anatomy* **110**, 103–124.

SUCHENWIRTH, R. & BUNDSCHU, H. D. (1970). Enzymhistologische Befunde an der Skeletmuskulatur des Menschen. I. Methoden und Ergebnisse bei Normalpersonen. *Klinische Wochenschrift* **48**, 1096–1101.

SUSHEELA, A. K. & GEORGE, J. C. (1963). Respiratory quotient of rat diaphragm. *Canadian Journal of Biochemistry and Physiology* **41**, 2221–2223.

SUSHEELA, A. K. & GEORGE, J. C. (1964). Fatty acid oxidation by rat diaphragm. *Journal of Animal Morphology and Physiology* **11**, 180–185.

SWANSON, M. A. (1948). Studies on the structure of polysaccharides. IV. Relation of the iodine color to the structure. *Journal of Biological Chemistry* **172**, 825–837.

TAKEUCHI, T. (1956). Histochemical demonstration of phosphorylase. *Journal of Histochemistry and Cytochemistry* **4**, 84.

TAKEUCHI, T. & KURIAKI, H. (1955). Histochemical detection of phosphorylase in animal tissues. *Journal of Histochemistry and Cytochemistry* **3**, 153–160.

TAKEUCHI, T. & SASAKI, M. (1968). Histochemical and electron microscopic differences between native glycogen and polyglucose synthesized by phosphorylase in tissue cells. *Acta histochemica et cytochemica* **1**, 63–78.

WACHSTEIN, M. & MEISEL, E. (1955). The distribution of histochemically demonstrable succinic dehydrogenase and of mitochondria in tongue and skeletal muscles. *Journal of Biophysical and Biochemical Cytology* **1**, 483–488.